Advancing treatment discovery for cognitive dysfunction in mood disorders with neuroimaging and psychological assessments – exemplified by erythropoietin

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Head of Faculty

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Evaluation committee
Max Gassmann, Professor
University of Zurich, Switzerland
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Steen Hasselbalch, Professor (Chair)
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University of Copenhagen, Denmark

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Dissertation for the Doctoral Degree in Medicine

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Psychiatric Centre Copenhagen
Rigshospitalet
University of Copenhagen
List of papers included in the dissertation

The dissertation is based on the following 12 papers:


Roman numbers in the compressed account of the research refer to these papers.
Preface

The studies in the dissertation were conducted from 2009-2016 during my appointments as postdoc researcher at the Copenhagen Affective Disorders Research Centre and clinical psychologist at the Copenhagen Clinic for Affective Disorders, Psychiatric Centre Copenhagen, Rigshospitalet.

I would like to express my gratitude to the people who have made this dissertation possible. First, I would like to thank my mentor, Professor Lars Vedel Kessing, MD, DMSc, for countless inspiring discussions, support and valuable advice. I also thank Senior Consultant Maj Vinberg, MD, PhD, for a fruitful collaboration and her commitment to the EPO trials without which they would not have been possible. The co-authors on the papers in the dissertation are thanked for their valuable scientific contributions. Many thanks go to my colleagues at the Copenhagen Clinic for Affective Disorders for their commitment to this research, including help with patient recruitment and assistance during holiday periods. I also thank my colleagues Klaus Munkholm, MD, DMSc, and Jens Bukh, MD, PhD, for their assistance with mood ratings in the EPO trials during my maternity leave and study nurses Susanne Sander and Hanne Nikolajsen for their great job with logistical planning and running the trials. I am grateful to the Copenhagen Clinic for Affective Disorders, headed by Senior Consultant Ellen Magrethe Christensen and Professor Lars Vedel Kessing, MD, DMSc, and to the Head of Department at Psychiatric Centre Copenhagen, Rigshospitalet, Ida Hageman, MD, for providing me with an inspiring and supportive environment with the necessary facilities and time for my research.

I would like to express my deep gratitude to the patients who have participated in this research. Without their time, effort and commitment, this research would not have been possible.

Finally, my warm thanks go to my husband, Maciej, for his continuous support and inspiring discussions and to my children, Anna, Oscar and Hector for the many precious distractions. I also thank my dad for his tireless encouragements and enthusiasm for my research and my mum for the loving care of her grandchildren, which enabled me to write up this dissertation.

Acknowledgements

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I. Why is cognition a future treatment target in mood disorders?

A. Mood disorders and functional disability
Unipolar disorder (UD) and bipolar disorder (BD) are common, persistent and often severely impairing mental disorders with a high degree of non-recovery, recurrence, and comorbidity (Grande et al. 2015; Kessler et al. 2003). Unipolar disorder and BD have an estimated lifetime prevalence of about 16% (Kessler et al. 2003) and 1-2% (Grande et al. 2015; Merikangas et al. 2007), respectively, and are among the leading causes of disability worldwide (Whiteford et al. 2013). These mood disorders are characterized by profound and persistent changes in affective states that result in substantial distress and functional impairment. Unipolar disorder is marked by low mood and/or anhedonia and associated symptoms including loss of energy, excessive guilt, sleep and psychomotor disturbances and cognitive symptoms for at least two consecutive weeks. Bipolar disorder involves periods of both depression and of mania or hypomania; discrete episodes with elated and/or irritable mood and related symptoms including increased energy and drive, reduced need for sleep and increased self-esteem. Patients with BD can also present mixed episodes in which depressive and manic symptoms are present simultaneously or fluctuate rapidly within the same episode. Mood disorders can thus be classified along a spectrum according to the extent and severity of mood elevation from UD to BD types II and I; UD is distinguished from BD by the absence of hypomanic, manic, or mixed episodes, while BD type II differs from BD type I by an absence of manic or mixed episodes. Mood disorders therefore differ mainly in their profile and severity of mood symptoms, which has led to growing consensus that a categorical distinction between the disorders is impossible (Goodwin et al. 2008).

A common feature of UD and BD is the substantial socio-occupational disability (Bonnin et al. 2010; Torrent et al. 2012; Tse et al. 2014), which constitutes the largest socio-economic burden of these disorders (Olesen et al. 2012; Wyatt and Henter, 1995). In particular, mood disorders cost an estimated €113 billion in Europe and $128 billion in the United States, of which reduced workforce capacity accounts for 60-80 % of the expenditures (Olesen et al. 2012; Wyatt and Henter, 1995). For UD, it has been estimated that 60 % of patients remain functionally disabled during periods of remission (i.e., periods where patients are relatively symptom-free), as reflected by a persistent reduction in work performance and psychosocial function (Jaeger et al. 2006). Further, two-thirds of the BD patients are unable to regain premorbid levels of social and occupational functioning even after a single mood episode (Huxley and Baldessarini, 2007) and their unemployment rates are 4- and 10-fold higher than in the general population (Huxley and Baldessarini, 2007; Kogan et al. 2004). Consequently, UD and BD are among the top 10 of all (mental and physical) illnesses with greatest impact on ‘days out of role’ (34 and 41 days per year for UD and BD, respectively) according to the WHO’s World Mental Health surveys (Alonso et al. 2011). Recent evidence indicates that cognitive deficits, including problems with memory, concentration and planning, are among the strongest
contributors - together with mood symptoms and illness progression - to patients’ functional disability and high unemployment rates (Bonnin et al. 2010; Torrent et al. 2012; Tse et al. 2014). Indeed, a close association has been observed between changes in cognition and socio-occupational functioning; patients who show cognitive improvement over the course of their treatment also tend to show improved life functioning, whereas those with no change or decline in cognitive function show deteriorated psychosocial functioning despite alleviation of depressive symptoms (Jaeger et al. 2006). This highlights the importance of targeting cognitive dysfunction to improve patients’ functional recovery – a major strategic focus for research in the next decade.

B. Functional disability and cognition
Cognition refers to a broad range of mental processes including attention, learning and memory, working memory, decision making and problem solving. While the analysis of cognitive deficits in schizophrenia is well established in the literature, cognitive dysfunction in BD and UD is a more recent field of scientific research. In the last decade, numerous studies in UD and BD have documented substantial cognitive deficits across memory, attention and executive function (Kurtz and Gerraty, 2009; Martinez-Aran et al. 2004; Rock et al. 2013). The profile of the cognitive impairment in mood disorders is highly similar to the pattern of deficits seen in schizophrenia, involving non-specific deficits across several domains (Barch, 2009; Etkin et al. 2013; Rund et al. 2006), although the degree of dysfunction varies between the disorders, being largest in schizophrenia (with effect sizes generally being >1 in schizophrenia and 0.5-0.7 in affective disorders) (Depp et al. 2007; Reichenberg et al. 2009; Schretlen et al. 2007) and larger in BD than in UD (Gualtieri and Morgan, 2008). Treatment of cognitive dysfunction may hence represent a common therapeutic approach across several neuropsychiatric disorders.

It is well-established that acute mood episodes are accompanied by broad cognitive deficits (Kurtz and Gerraty, 2009; Martinez-Aran et al. 2004; Rock et al. 2013). However, it is still unclear whether these deficits are merely secondary to mood symptoms or, in contrast, constitute a separate illness dimension with separate evolution, prognosis, and impact on functional status (Murrough et al. 2011). Evidence from longitudinal and meta-analytic studies strongly indicates the latter, documenting cognitive deficits before onset of illness and persistence of cognitive dysfunction beyond acute mood episodes. In particular, meta-analytic evidence has shown mild to moderate cognitive dysfunction in individuals at genetic risk for mood disorders (Bora et al. 2009), which increases their risk of developing a psychiatric disorder as shown in a recent study by our group (Vinberg et al. 2013). Consistent with this, trait-related cognitive deficits across several domains have been observed during remission already at BD illness onset and in recurrent UD (Bora et al. 2013; Bourne et al. 2013) and are more pronounced at later illness stages (Rosa et al. 2014). These emerging findings indicate that cognitive deficits may reflect both genetic abnormalities and neurotoxic effects of mood episodes.
This ‘cognitive neuroprogression hypothesis’ remains controversial due to a lack of large-scale long-term longitudinal trials but is, nevertheless, consistent with evidence for (i) more pronounced cognitive dysfunction in patients with a history of psychosis (Bora et al. 2010), (ii) larger grey matter volume reduction in patients with longer illness duration (Gildengers et al. 2014), (iii) and a positive correlation between the number of affective episodes, the severity of cognitive deficits (Lopez-Jaramillo et al. 2010; Martinez-Aran et al. 2004) and the risk of dementia (Kessing and Andersen, 2004).

Cognitive dysfunction is not only associated with decreased socio-occupational capacity but also with prolonged illness duration and reduced chances of recovery, independently of mood symptoms (Etkin et al. 2013). The underlying link between cognitive deficits, poor prognosis and socio-occupational disability may be both direct and indirect. Cognitive difficulties may, for example, directly limit patients’ ability to focus and remember appointments at work and impede patients’ ability to profit from psychological interventions. They may also indirectly lead to poorer prognosis because of their negative effects on decision making, which can lead to more stressful life events and thereby greater risk of depressive or manic relapse. Taken together, the evidence that cognitive dysfunction is partially separate from mood symptoms and has direct impact on prognosis and socio-occupational outcome highlights cognitive dysfunction as an emerging treatment indication in mood disorders. Notwithstanding the pressing need to improve cognition, there are no available treatments for cognitive dysfunction in these patients. Progress is therefore urgently needed in treatment development targeting cognition in mood disorders to enhance patients’ functional recovery and reduce the associated societal costs.

C. Cognitive dysfunction and neuroplasticity
The neurobiological underpinnings of the cognitive deficits in mood disorders remain elusive. However, converging evidence from preclinical studies, neuroimaging studies, and postmortem studies of patients with mood disorders suggests that they arise from disruption of neuroplasticity and structural changes in brain regions including the hippocampus and related neural circuits. In particular, magnetic resonance imaging (MRI) of UD and BD patients in both depressed and remitted phases and postmortem immunohistochemical studies have documented reduction in overall hippocampal volume (Canales-Rodriguez et al. 2013; McKinnon et al. 2009) and in hippocampal subregions including the dentate gyrus (DG), cornu ammonis CA1-3 region, and subiculum (Cole et al. 2010; Elvsashagen et al. 2013; Huang et al. 2013; Lucassen et al. 2001). Preclinical studies suggest that the hippocampal volume reduction is caused by dendritic retraction in the CA1-3 region and DG, CA3 pyramidal cell death, and suppression of DG neurogenesis due to glucocorticoid overexposure (Alfarez et al. 2008; Czeh and Lucassen, 2007; Hageman et al. 2008). In keeping with this, postmortem examination of hippocampi of depressed individuals has revealed loss of dendritic branching, dendritic spine complexity, and glia (Cobb et al. 2013; Stockmeier and Rajkowska, 2004) but no substantial neural atrophy or
suppressed DG neurogenesis (Czeh and Lucassen, 2007; Lucassen et al. 2001). These neuroplasticity deficits have negative consequences for cognition. In particular, animal studies have demonstrated disruption of synaptic plasticity mechanisms such as long-term potentiation (LTP) in the hippocampus and PFC is associated with learning and memory deficits and impaired behavioural flexibility (Marsden, 2013) which is reminiscent of the pervasive problems with memory and decision making in patients with mood disorders.

Conversely, restoration of neuroplasticity is a putative common mechanism of long-term antidepressant or mood stabilizing drug treatment (Berton and Nestler, 2006; Duman et al. 1999; Manji et al. 2003). For example, antidepressant drug therapy in UD has been shown to increase the volume of the left hippocampus after three years (Frodl et al. 2008) and of bilateral hippocampi after eight years of treatment (Hviid et al. 2010). Further, lithium produces bilateral increase in hippocampal volume in BD after eight weeks (Yucel et al. 2008) and four years of treatment (Yucel et al. 2007). Such increase in neuroplasticity also seems to underlie the hippocampal volume increase after electroconvulsive therapy (ECT) (Abbott et al. 2014; Jorgensen et al. 2015), although paradoxically this treatment is known for its adverse effects on cognition (Fraser et al. 2008; Semkovska and McLoughlin, 2010) which are still controversial (Semkovska and McLoughlin, 2010). The treatment-associated volumetric changes at the macroscopic level are likely to result from increase in neurotrophic factors including brain-derived neurotrophic factor (BDNF) in the hippocampus, dendritic branching and other cellular plasticity mechanisms (Castren and Rantamaki, 2008; Manji et al. 2000; Shim et al. 2013). Together, the findings support the idea that direct and enduring upregulation of neuroplasticity is a fruitful treatment approach to target not only mood symptoms but also cognitive dysfunction in mood disorders.

D. Aims and structure of the dissertation

The three overarching aims of the dissertation are:

1. To delineate and discuss the major methodological challenges that impede the success of treatment discovery targeting cognition in mood disorders.

2. To exemplify with the author’s line of EPO research how directly targeting neuroplasticity may improve cognition in mood disorders and outline key methodological lessons learnt from this research with implications for the field.

3. To identify feasible, sensitive and valid psychological assessment tools to screen for cognitive impairment in future cognition trials in mood disorders to ensure enriched patient samples with scope for cognitive improvement.

The dissertation is based on 12 published papers: A systematic review of cognition trials in BD (paper I), a systematic review of preclinical and human fMRI studies of EPO to target cognitive impairment (paper II), the published study protocol of the author’s randomised controlled EPO
trials (paper III), seven papers delineating the clinical and neuroimaging results from these trials (papers IV-X), and two papers that validate new psychological screening tools for cognitive dysfunction in UD and BD (papers XI-XII).

The dissertation is structured into six chapters: Chapters I-V build on the above articles and address the following questions: Why is cognition a future treatment target in mood disorders? What are the methodological challenges and future directions in cognition trials? How can neuroimaging aid treatment discovery? How can we identify an fMRI biomarker for cognitive enhancement? Why is erythropoietin a promising cognition treatment? What can we learn from the erythropoietin trials in mood disorders? Which psychological assessments should we use to screen for cognitive impairment? Finally, chapter VI is a general discussion that summarizes the methodological challenges in the field, evaluates the contributions and limitations of the present research, discusses opportunities and challenges with implementation of fMRI in treatment development targeting cognition and highlights future directions for the field.
II. What are the methodological challenges and future directions in cognition trials?

This chapter is partially based on paper I.

A. Cognition trials in bipolar disorder

The association between neuroplasticity and a wide range of changes in neurotrophin levels, neurogenesis, metabolism, neurotransmitters (such as acetylcholine, dopamine and glutamate), inflammation, oxidative stress and cortisol has encouraged intensive research over the past decade into the efficacy of interventions that act on some or multiple of these pathways to increase neuroplasticity in mood disorders. Overall, the findings are contradictory and unclear.

We have therefore conducted a systematic review with Cochrane risk of bias evaluations of randomised controlled trials (RCTs) and open label studies of novel pharmacological and psychological treatments for cognitive dysfunction in BD (paper I). A total of 19 studies were identified of which 13 (11 pharmacological and two psychological intervention trials) were randomised and controlled.

In brief, the pharmacological studies revealed no cognitive benefits of the following compounds: the cholinesterase inhibitor donepezil (Gildengers et al. 2008), the prodrug for L-cysteine N-acetyl cysteine (Dean et al. 2012), the dopamine agonist pramipexole (Burdick et al. 2012) or the endogenous steroid hormone pregnenolone (Osuji et al. 2010). The precursor of phosphatidylcholine citicoline improved verbal memory in one study (Brown et al. 2007) but not in another trial (Brown and Gabrielson, 2012), thus rendering the evidence inconclusive. Some support for pro-cognitive effects were found for the corticosteroid receptor antagonist mifeprisone on spatial working memory (Watson et al. 2012; Young et al. 2004), of the cholinesterase inhibitor galantamine on verbal memory (Ghaemi et al. 2009; Iosifescu et al. 2009), of intranasal insulin on executive function (McIntyre et al. 2012), and of the herbal medicine withania somnifera on processing speed, working memory and social cognition (Chengappa et al. 2013). However, a key limitation of the studies was that the observed effects would not have survived Bonferroni correction for multiple testing in all cases but one due to lack of a priori priority between the cognition outcomes (i.e., selection of primary, secondary and tertiary outcomes). Finally, the multifunctional growth factor erythropoietin (EPO) improved sustained attention, memory and executive function (eight of the 15 cognition outcomes), of which the effect on sustained attention (secondary outcome) would have survived Bonferroni correction if adjusted for all 15 cognition measures. Nevertheless, the findings must be regarded as preliminary as there was only a trend toward EPO-associated improvement of the primary outcome (verbal memory).

Of the psychological intervention studies, four investigated cognitive remediation (CR) (Deckersbach et al. 2010; Demant et al. 2015b; Meusel et al. 2013; Naismith et al. 2010), one investigated computerized cognitive training (Preiss et al. 2013) and one examined
functional remediation (FR) in BD or mixed UD-BD samples (Torrent et al. 2013). Two small non-randomised and/or non-controlled CR trials showed CR-related improvement in memory (Naismith et al. 2010) and observer-based executive function (i.e., not an objective neuropsychological measure) (Deckersbach et al. 2010). However, a subsequent RCT from our group revealed no cognitive benefits of short-term CR in remitted BD (Demant et al. 2015b). Computerized cognitive training showed some training-related improvement in executive function in a non-randomised trial (Preiss et al. 2013) but the effects would have not survived Bonferroni correction and no adjustments were made for change in mood symptoms (the co-primary treatment aim). Finally, FR showed no cognitive benefits in a large multi-centre RCT in partially remitted BD patients (although improvements were observed in functional capacity, the primary trial outcome) (Torrent et al. 2013).

In general, the sample sizes of the RCTs in BD were small: two studies had ≤10 participants per group, 10 studies included 12-34 participants per group and only one multicenter trial (in which cognition was secondary outcome) had a large sample with 77-82 participants per group (for details, see paper I). Further, the risk of bias was deemed high nine of the 13 (69%) randomised, controlled studies and low in only three studies (23%) (see Table 1). Key methodological problems were insufficient details on how allocation was concealed in nine (69%) trials, inadequate information on how knowledge of allocation was prevented in 11 (85%) trials and potential for selective outcome reporting (given no a priori priority between outcomes or Bonferroni corrections) in eight (62%) trials (Table 1). In eight (62%) studies there were also other potential sources of bias, including large drop-out rates (with no statistical adjustment for missing data) and lack of details on statistical adjustment for changes in mood, which could drive any observed effects on cognition (for details, see paper I). Taken together, the RCTs of candidate treatments in BD suffer from several methodological problems and, consequently, the risk of bias in the field is high.

Table 1. Risk of bias in the randomised controlled trials in BD assessed with the Cochrane Collaboration’s Risk of Bias tool. The table is a modified version of table 2 in Miskowiak et al, Eur Neuropsychopharmacology, in press (paper I).

<table>
<thead>
<tr>
<th>Study</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Global judgement</th>
</tr>
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<tr>
<td>Brown et al. (2007) (Brown et al. 2007)</td>
<td>Yes</td>
<td>Unclear</td>
<td>Unclear</td>
<td>No</td>
<td>No</td>
<td>No; low completion rate</td>
<td>High</td>
</tr>
<tr>
<td>Brown et al. (2012) (Brown and Gabrielson, 2012)</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Yes</td>
<td>Unclear</td>
<td>No; low completion rate</td>
<td>High</td>
</tr>
<tr>
<td>Burdick et al. (2012) (Burdick et al. 2012)</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Yes</td>
<td>No</td>
<td>No; not intention-to-treat analysis</td>
<td>High</td>
</tr>
<tr>
<td>Chengappa et al. (2013) (Chengappa et al. 2013)</td>
<td>Yes</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>High</td>
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<tr>
<td>Dean et al. (2012) (Dean et al. 2012)</td>
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<td>Unclear</td>
<td>Unclear</td>
<td>No</td>
<td>No</td>
<td>No; only a subgroup with cognition data from larger study tested</td>
<td>High</td>
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<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>High</td>
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<tr>
<td>Ghaemi et al. (2009) (Ghaemi et al. 2009)</td>
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<td>Unclear</td>
<td>Unclear</td>
<td>No</td>
<td>No</td>
<td>No; low completion rate</td>
<td>High</td>
</tr>
<tr>
<td>Study/Year</td>
<td>Allocation</td>
<td>Allocation Concealment</td>
<td>Knowledge of Allocation</td>
<td>Incomplete Outcome Data</td>
<td>Free of Selective Outcome Reporting</td>
<td>Treatment Effect on Cognition</td>
<td>Risk of Bias</td>
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<tr>
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<td>Unclear</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
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<tr>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>Unclear</td>
<td>Unclear</td>
<td>No</td>
<td>No</td>
<td>Unclear: given the priority of cognition as secondary outcome</td>
<td>High</td>
</tr>
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<td>Torrent et al. (2013) (2013)</td>
<td>Yes</td>
<td>Unclear</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Unclear: no description of whether cognitive change would be adjusted for change in depression symptoms</td>
<td>Low</td>
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<tr>
<td>Watson et al. (2012) (2012)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Unclear</td>
<td>Low</td>
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</tbody>
</table>


**B. Cognition trials in unipolar disorder**

Antidepressant drug treatments *indirectly* alleviate cognitive dysfunction as a consequence of decrease in mood symptoms (i.e., pseudospecific effects) (Rosenblat et al. 2015). However, in a recent review we found a paucity of evidence for any *direct* effects of treatments on cognition (Bortolato et al. 2016). In particular, randomised, controlled studies of galantamine showed no cognitive benefits (Elgamal and Macqueen, 2008; Holtzheimer, III et al. 2008), while the d-amphetamine prodrug lizdexamfetamine dimesylate and the major methyl-donor s-adenosyl methionine reduced subjective cognitive difficulties (Levkovitz et al. 2012; Madhoo et al. 2014). The pleiotropic agent modafinil, which was originally intended to treat narcolepsy, improved executive function (but no other aspects of cognition) in small open-label trial (DeBattista et al. 2004). Notably, *pseudospecificity* cannot be ruled out in these trials given concomitant improvements in mood that were not controlled for in the statistical analyses. A recent RCT from our group revealed *mood-independent* long-lasting beneficial effects of EPO on verbal learning and memory in treatment-resistant symptomatic UD (Miskowiak et al. 2014b). However, the finding must be considered preliminary since cognition was only defined as tertiary outcome in the trial (with mood symptoms being the primary treatment target) (Miskowiak et al. 2014b).

Recently, the new multimodal antidepressant vortioxetine was shown in two RCTs to have positive effects on some aspects of cognition in symptomatic UD that seem to be partially independent of its effects on mood as evidenced by statistical 'path analyses' (Mahableshwarkar et al. 2015; McIntyre et al. 2014). In a subsequent (not yet published) study in remitted UD, beneficial effects of vortioxetine were also found on psychomotor speed (but not on memory or executive function), suggesting that the cognitive effects are at least partially mood-independent (Smith et al. 2016). Meta-analysis of nine CR studies in UD also found some evidence for treatment effects on attention and working memory (but not on memory or executive function), although again pseudospecificity cannot be ruled out given concomitant treatment-related improvements in mood (Motter et al. 2016).
In conclusion, RCTs of candidate treatments in UD have revealed only preliminary effects of new candidate cognition treatments. These trials are often characterised by similar methodological challenges to those observed in BD cognition trials, including lack of consensus on which cognition outcomes to select as primary, how to adjust for multiple testing and how to control for concomitant treatment-related change in mood symptoms. These general methodological problems must be addressed to improve future cognition trials in mood disorders.

C. The major methodological challenges
A general limitation in the field is the lack of consensus or guidelines for the design of cognition trials in mood disorders despite the methodological problems and high risk of bias in most studies in the field. Specifically, (i) there is no agreement upon the need to screen for cognitive impairment in these trials (with only one fifth of studies including a screening for cognitive impairment) and whether screening criteria should be subjective or objective (i.e., self-rated or performance-based). (ii) It is also unclear which cognitive measure(s) should be employed to track treatment efficacy and whether/how these should be prioritised. Other unresolved questions are (iii) whether trial participants may present current mood symptoms or should be euthymic (i.e., completely symptom-free), (iv) how concomitant antidepressant or mood-stabilizing medication should be managed, (v) whether cognition treatments should target patients at certain illness stages, and (vi) what statistical methods should be employed. The absence of a clear consensus regarding these issues is a serious limitation that must be addressed to advance the success rates of cognition trials in mood disorders. This section provides and in-depth discussion of the methodological issues regarding screening for cognitive impairment and selection of cognition outcomes to track treatment efficacy and touches briefly on the additional difficulties in the field.

1. Screening for cognitive impairment
In our systematic review of cognition trials in BD (paper I), the vast majority of studies (79%) used no screening for cognitive impairment. Of the studies that did include screening for cognitive impairment, all but one applied subjective criteria. For schizophrenia trials, there is generally no need to screen for cognitive impairment since almost all patients display broad cognitive deficits with large effect sizes (Schaefer et al. 2013). In contrast, patients with mood disorders tend to show less severe deficits (Bourne et al. 2013) and substantial cognitive heterogeneity, with approximately 30-50% of BD patients (Burdick et al. 2014; Jensen et al. 2016) and 60-80% of UD patients being relatively cognitively intact (Gualtieri and Morgan, 2008) despite their frequent subjective cognitive complaints. This inevitably introduces risk of enrolling UD and BD patients with no objective cognitive dysfunction and hence little scope for cognitive improvement, which increases type II errors (Bonnin et al. 2015; Burdick et al. 2012). Even if
there were consensus on the need to screen for cognitive impairment before enrolment of patients in cognition trials, the question still remains how we best screen for cognitive impairment: can we rely on patients’ subjective self-reported cognitive difficulties or are objective neuropsychological tools necessary?

The use of subjective screening criteria has several advantages: first, it is important for recruitment and treatment adherence purposes that the patients experience cognitive difficulties to be motivated for taking part in the study and do not drop out despite the demands on time and effort. Second, it is easy to just ask patients whether they experience cognitive difficulties or give them a self-report questionnaire. Until recently, the general perception has been (and is still to some degree) that subjective and objective measures of cognitive function reflect the same thing; hence, if a patient experiences cognitive problems this is thought reflect real objective impairment. Nevertheless, recent research from our and other groups has documented a remarkably poor correlation between subjectively reported cognitive difficulties and objective cognitive performance deficits in mood disorders (Demant et al. 2015a; Martinez-Aran et al. 2005; Svendsen et al. 2012). While patients displayed both objective and subjective cognitive impairment relative to healthy controls, the correlation between the degree of objective and subjective difficulties was weak or absent (Demant et al. 2015a; Martinez-Aran et al. 2005; Svendsen et al. 2012). It is therefore not necessarily the patients with the greatest objective cognitive dysfunction who experience the most pronounced cognitive problems, and vice versa. In particular, the relation between objective and subjective measures of cognition is modified by factors like affective symptoms (and associated negative self-evaluations), as demonstrated in several of our studies (Demant et al. 2015a; Jensen et al. 2015) and as reported by Martinez-Aran and colleagues (Martinez-Aran et al. 2005) who also identified comorbidity and medication side effects as major causes of subjective impairment. This calls into question the validity of the subjective assessments for investigation of objective cognitive function.

It has been suggested that subjective cognition measures may better capture cognitive decline in patients with supra-normal premorbid function (Forcada et al. 2015). However, we recently found that greater cognitive reserve, as reflected by higher verbal IQ, was associated with disproportionately fewer subjective than objective cognitive difficulties (i.e., a tendency to ‘under-report’ cognitive deficits) (Miskowiak et al. 2016). Nevertheless, such patients could still be quite disabled due to their cognitive decline in work settings, particularly if their job involves multi-tasking, strategic planning and fast decision making. Conversely, more subjective than objective cognitive impairment was associated with more sub-syndromal depression and mania symptoms, BD type II diagnosis, more previous hospitalisations and male gender (Miskowiak et al. 2016). Given this, objective screening seems warranted for correct detection of cognitive impairment in patients with high IQ and in patients with substantial residual mood symptoms, a severe illness course and/or BD type II. Nevertheless, the necessity to screen for cognitive
impairment is still an unresolved issue in trials targeting cognition in mood disorders, with serious implications for the success rates of these trials.

2. Selection of treatment efficacy outcomes
Another key challenge in the field is the lack of clarity on how to best track treatment efficacy on cognition in mood disorders. It could be argued that cognition treatments are only clinically meaningful if patients experience treatment-related cognitive improvement. However, the poor correlation between objective and subjective cognition measures (Miskowiak et al. 2014a) indicates that patients may be unable to correctly assess their own cognitive capacity or cognitive change. Objective measures of cognition are therefore essential to directly capture treatment-induced changes in cognition. Accordingly, all BD cognition trials in our systematic review but one (Deckersbach et al. 2010) used objective neuropsychological tests for investigating treatment-associated changes in cognition. Despite the direct impact of cognitive impairment on socio-occupational impairment (Mur et al. 2009; Tse et al. 2014), cognitive performance is often regarded as a ‘technical’ outcome with inadequate ecological validity since it can only account for 10-45 % of variance in the daily life skills, depending on the particular neuropsychological test (Chaytor and Schmitter-Edgecombe, 2003). This is likely to result from inherent problems with demonstrating ecological validity of neuropsychological tests since these are conducted in a highly structured setting with specific instructions and no distractions which has little resemblance to daily life situations. Nevertheless, studies have generally found a moderate degree of correlation between daily functioning and neuropsychological performance (which is highest for memory tests) (Chaytor and Schmitter-Edgecombe, 2003), indicating that cognitive test performance is a valid albeit not perfect proxy of functional capacity. One approach to overcome the ecologic validity issue with neuropsychological tests in cognition trials has been to select a socio-occupational measure as primary treatment outcome (e.g., (Torrent et al. 2013)). However, this approach has the caveat that several additional factors contribute to socio-occupational disability including depressive symptoms, life-style, personality and psychosocial circumstances. Hence if the aim of a trial is to clarify whether a candidate treatment exerts pro-cognitive effects, it is necessary to select an objective cognition measure as primary outcome. A functional measure may then be included as co-primary or key secondary outcome to also capture the functional implications of potential cognitive improvement.

The next challenge is the selection of an objective cognition outcome for tracking treatment efficacy in patients with mood disorders. It is still an unresolved question whether treatment efficacy should be measured with a single (global or domain-specific) or multiple cognition outcomes. Indeed, the majority of cognition trials in BD and UD investigated multiple cognition outcomes with no a priori defined hierarchy between them. In general, these studies did not apply Bonferroni corrections for multiple testing which renders high risk of bias due to the potential for selective outcome reporting. This has turned out to be a general problem in clinical
trials across several fields and has therefore been addressed in the CONSORT (Consolidated Standards for Reporting Trials) statement, a widely implemented guideline for reporting RCTs (Moher et al. 2012). To adhere to the CONSORT criteria, cognition trials must prioritize the cognition measures into primary, secondary and tertiary outcomes, respectively, and also state the primary time for these endpoint assessments. If the primary outcome turns out negative at the primary assessment time, the trial must be deemed overall negative. This predetermined priority between outcomes in clinical trials safeguards against post-hoc distortive claims. It is also an advantage that Bonferroni correction should only be applied within each outcome level; i.e. if a single primary outcome measure is selected, the statistical threshold for detection of a treatment-related effect is typically $P=0.05$, even if the study has multiple secondary outcomes, while selection of two co-primary outcomes would reduce the alpha-level to $P=0.05/2=0.025$.

This approach has been used in only a subset of cognition trials in mood disorders to date, but its implementation is critical for future trials.

Consensus is needed with respect to which particular objective cognition measure(s) should be used as primary outcomes in cognition trials in mood disorders and whether the primary outcome should be a single neuropsychological test (or subtest measure) or, in contrast, a global cognitive composite score summarizing the performance across several domains. The MATRICS Consensus Cognitive Battery (MCCB) is a standard test battery for cognition trials in schizophrenia, with the primary outcome being change in an overall MCCB composite score (Kern et al. 2008; Nuechterlein et al. 2008). No such consensus battery has yet been developed for mood disorders. It has been suggested that the MCCB with addition of some higher-order (more complex) verbal learning and executive function tests could be suitable for assessment of cognition in BD given the cognitive overlap between BD and schizophrenia (Yatham et al. 2010). Indeed, recent empirical investigations suggest that the MCCB (with some additional executive function tests) is sensitive to cognitive deficits in BD (Van Rheenen and Rossell, 2014) and in TRD (Mohn and Rund, 2016). However, the cognitive domains in the MCCB are derived from schizophrenia research and may therefore not be optimal for UD and BD given potential differences in the factor structure of cognitive impairments in these disorders (Bora and Pantelis, 2011). Additional systematic research including factor analytic studies of cognitive impairments in UD and BD therefore seems necessary to first identify the most useful cognitive tests and domains, after which validation studies of a proposed battery can be conducted (Bora and Pantelis, 2011). Given the current lack of a consensus battery for mood disorders, trials have used various different cognition measures which may partially explain the heterogeneous findings.

3. Other methodological challenges

Mood state – It is well-known that mood symptoms impact on cognitive performance. Trials that include patients in symptomatic states and show improvement of both cognition and mood symptoms are therefore unable to rule out pseudospecificity. Mood symptoms may also mask
potential efficacy of an intervention for cognitive dysfunction (Burdick et al. 2012). Nevertheless, sub-syndromal symptoms should be allowed for two reasons; first, inclusion of only patients in full remission would render an unrepresentative patient sample and thereby limit the generalizability of the findings. Second, such strict criteria would reduce enrolment feasibility and consequently the study sample sizes. Given this, cognition trials in mood disorders should recruit both fully and partially remitted patients who are clinically stable; mood symptoms should then be assessed regularly throughout the trial and should be controlled for in the outcome analyses, similar to the approach in our EPO trials (Miskowiak et al. 2014a; Miskowiak et al. 2014b).

Role of concomitant medication – For ethical reasons and for the effects of the cognition treatments to be representative of the actual clinical practice, the investigational cognition treatments should be delivered as add-on to patients’ usual medication. However, since antidepressant, mood-stabilizing and antipsychotic medication may have adverse actions on cognition (Dias et al. 2012), concomitant mediation could potentially mask or, alternatively, enhance the effects of the investigational compound (Burdick et al. 2015). One way to tackle this problem is to ensure that the concomitant medication is within the recommended dose range (for lithium and antipsychotics, in particular) from a couple of weeks before trial start and that the medication is carefully recorded and kept stable throughout the study, if possible. Post-hoc analyses are also warranted to explore any potential interaction effects of patients’ medication and the investigational compound, as exemplified in our EPO studies (Miskowiak et al. 2014a; Miskowiak et al. 2014b; Miskowiak et al. 2015a).

Staging – Mood disorders are hypothesized to involve ‘clinical staging’, a progression from prodromal (at-risk) to more severe and resistant presentations (Cosci and Fava, 2013; Vieta et al. 2011). This idea is based on the observation that treatment response is generally better when introduced early in the course of illness (Kessing et al. 2014) and assumes that earlier stages require simpler interventions (Vieta et al. 2011). In line with the staging model, it is conceivable that the effect of the interventions for cognitive dysfunction may differ between disease states (Grande et al. 2015; Vieta, 2015). Inclusion of a heterogeneous group of patients at different illness stages could therefore mask potential pro-cognitive effects of a particular intervention, especially if sample sizes are small. Future studies are therefore warranted to clarify the influence of illness stage on the chances for treatment efficacy on cognition.

In conclusion, the overall disappointing findings in the field are partially due to high risk of bias in most cognition trials. Several critical methodological challenges must be addressed for advances to be seen in the field, including the issues regarding the need to screen for cognitive impairment and whether to use subjective or objective screening criteria, as well as regarding which primary cognition outcome measure(s) to select for the assessment of treatment efficacy. In addition, treatment discovery faces another more fundamental methodological problem: the lack of insight into whether the candidate cognition treatments under investigation actually target the aberrant
function in cognition-relevant neurocircuitries that underlies patients’ cognitive deficits. To gain insights into such potential neurocircuitry ‘target engagement’, neuroimaging techniques should be implemented in future drug discovery strategies.

D. How can neuroimaging aid treatment discovery?

1. Neuroimaging and CNS drug discovery
Drug development for central nervous system (CNS) conditions has been hampered by high failure rates. In particular, only 15% of new chemical entities with CNS indication are eventually filed for approval in an ‘Investigational new drug application’ to the Food and Drug Administration (FDA) (Borsook et al. 2013; DiMasi, 2001). Many large pharmaceutical companies have therefore abandoned the field, the so-called ‘death of CNS drug development’, which has led to a large unmet clinical need for new CNS treatments (Cutler, 2011). Most of these drug discovery programs – including those targeting cognition - have not used a brain ‘circuit-based’ approach and hence had no information about whether the drugs modulated the neural circuits of interest. The high failure rates are therefore possibly due to the drugs not effectively engaging the key neural circuitries, which is important to establish prior to clinical phase 3 RCTs (Borsook et al. 2013; Nathan et al. 2014). In particular, drug screening typically relies on animal models and if beneficial effects are seen, the compounds are moved directly into large, costly clinical efficacy phase 3 trials. However, discovery of therapeutic-like effects of a compound in animal models has turned out to provide poor prediction of its efficacy in clinical populations (Millan et al. 2012). A key priority for neuropsychiatric research is therefore the identification of sensitive brain-based biomarker models that can act as ‘surrogate endpoints’; biomarkers that predict and can thereby serve as interim evidence for treatment efficacy (Biomarkers Definitions Working Group, 2001).

Consistent with this, the FDA Critical Path Initiative has recently highlighted neuroimaging in human populations as a key tool to accelerate the screening and selection of new candidate CNS treatments (Food and Drug Administration, 2016). In particular, application of a neuroimaging biomarker model in treatment discovery targeting cognition carries the potential to identify early change in key neuronal networks that predicts subsequent cognitive improvement. Such detection of such ‘therapeutic-like’ effects on cognition could guide the development of new mechanism compounds for cognitive dysfunction as a conceptually important middle step between investigation of novel treatments in animal models and large-scale clinical phase 3 trials (Nathan et al. 2014).

2. Use of fMRI in antidepressant drug screening
Functional MRI provides a useful indirect marker of neuronal activity through the measurement of blood-oxygen-level dependent (BOLD) activity (Ogawa et al. 1992) and is therefore
increasingly applied to get insight into the functional mechanisms of action of drugs and as a surrogate marker of drug efficacy in CNS conditions. Specifically, application of fMRI in treatment development can elucidate the functional effects of treatments at a ‘systems level’ in the brain rather than at a neurochemical or receptor level (Nathan et al. 2014). Thereby, fMRI provides a common ‘downstream’ circuitry-based biomarker for neurochemically different treatments that can restore neurocircuitry function. In phase 1 and 2 studies, fMRI may therefore demonstrate ‘target engagement’ in the key neural circuits as a first step in establishing proof-of-mechanism and proof-of-concept (Nathan et al. 2014).

An illustrative example of this approach is the development of neurocognitive biomarker models for antidepressant and anxiolytic drug action. Negative cognitive bias in attention and memory in depression and anxiety disorders is linked to impaired prefrontal top-down regulation of exaggerated activity in limbic regions including the amygdala (Miskowiak and Carvalho, 2014). This imbalance that is normalized by various efficacious antidepressant and anxiolytic compounds with different neurochemical mechanisms (i.e., selective serotonin/norepinephrine reuptake inhibitors) and by effective psychological interventions, which predicts subsequent clinical outcome (Harmer, 2006; Harmer et al. 2010; Harmer and Cowen, 2013; Nathan et al. 2014; Phan et al. 2013; Reinecke et al. 2014; Tranter et al. 2009). In contrast, non-effective treatments fail to show such consistent effects on neurocognitive measures of emotional processing (Chandra et al. 2010; McCabe et al. 2009; Pringle et al. 2011). Modulation of behavioural and cortico-limbic response during emotional processing therefore seems to be valid biomarker models for antidepressant and anxiolytic effects (Nathan et al. 2014), and may provide a more accurate prediction of treatment efficacy than animal models (Hafizi et al. 2007). This has led to an increasing recognition in the pharmaceutical industry of the value of neurocognitive biomarker models for antidepressant and anxiolytic drug action as proof-of-concept in the early screening of novel candidate treatments (Nathan et al. 2014).

E. How can we identify an fMRI biomarker model for cognitive enhancement?

In contrast with fMRI studies of treatments for depression and anxiety symptoms, there is a paucity of fMRI studies of candidate treatments for cognitive dysfunction. One explanation may be that cognitive dysfunction is itself commonly regarded as a biomarker of pathological brain conditions rather than a treatment target per se. Another possible explanation is that effective cognition treatments are a prerequisite for identification of a valid circuitry-based biomarker model for cognitive enhancement. Specifically, a valid biomarker model for cognitive enhancement must fulfil five key validity criteria: it must (i) be sensitive to a treatment with pro-cognitive effects, (ii) produce similar effects in patients with cognitive dysfunction and healthy participants, (iii) be sensitive to effective treatments with different neurochemical mechanisms, (iv) be unresponsive to ineffective treatments, and (v) be sensitive to both cognitive improvement and –decline (Harmer et al. 2010). Based on these criteria, it is difficult to evaluate criteria (i)-(iii) in the absence of any established efficacious treatments for cognitive
dysfunction. In fact, there seems to be a quandary: efficacious cognition treatments are needed to validate a circuitry-based biomarker model for cognitive enhancement, and a valid circuitry-based biomarker model for cognitive enhancement is needed to identify new effective cognition treatments.

A potential solution to the problem is a step-wise approach with which we: (i) identify the most reliable functional neuronal correlates of cognitive deficits in neuropsychiatric disorders, (ii) select one of the most promising candidate treatments and test its ability to modulate the activity in these dysfunctional neural circuitries in a short-term proof-of-concept fMRI study, and (iii) if target engagement is shown in (ii), then test the effects of this candidate treatment in a longer-term clinical phase 2 trial in patients using fMRI to elucidate the neuronal changes underlying potential pro-cognitive effects. Together, these three steps should provide the first key insights into a putative circuitry-based biomarker model for cognitive enhancement. Additional studies would then be necessary to validate the identified candidate biomarker model against the general biomarker model validity criteria.

Regarding point (i), a common functional neuronal correlate for cognitive dysfunction across distinct neuropsychiatric disorders is aberrant activity in the prefrontal cortex (PFC) and related neural circuitries during performance on working memory and episodic encoding tasks (Dietsche et al. 2014; Fernandez-Corcuera et al. 2013a; Frangou et al. 2008; Hamilton et al. 2009; Minzenberg et al. 2009; Monks et al. 2004; Ragland et al. 2009; Townsend et al. 2010). Specifically, fMRI studies indicate that patients’ memory deficits arise from aberrant neural activity in the medial and dorsolateral PFC (mPFC and dlPFC) and medial temporal regions including the hippocampus during symptomatic phases (Dietsche et al. 2014; Fairhall et al. 2010; Kelley et al. 2013; Werner et al. 2009) and after remission from mood episodes (Glahn et al. 2010; Hall et al. 2010; Milne et al. 2012). Hippocampal hypo-activity has been observed during memory encoding and retrieval in UD (Fairhall et al. 2010; Kelley et al. 2013; Milne et al. 2012; Werner et al. 2009) and during retrieval in BD (Glahn et al. 2010), although two studies found no hippocampal activity change (Avery et al. 2013; Werner et al. 2009). Interestingly, encoding-related hippocampal response correlates with recall accuracy in healthy but not in UD or BD individuals (Dietsche et al. 2014; Fairhall et al. 2010; Glahn et al. 2010), pointing to deficient hippocampal recruitment during memory formation in mood disorders. The majority of studies also report hypo-activity in the mPFC and/or dlPFC during memory encoding (Dietsche et al. 2014; Hall et al. 2010; Kelley et al. 2013) and in the dlPFC during retrieval (Glahn et al. 2010; Kelley et al. 2013). Executive dysfunction in mood disorders is most consistently associated with dlPFC hypo-activity, which is evident during working memory performance in symptomatic phases (Fernandez-Corcuera et al. 2013a; Garrett et al. 2011; Siegle et al. 2007; Townsend et al. 2010) and remission (Pomarol-Clotet et al. 2015; Townsend et al. 2010), although notably a few studies observed dlPFC hyper-activity (Harvey et al. 2005; Matsuo et al. 2007). The aberrant dlPFC activity is often accompanied by a failure to
suppress activity in the default mode network (DMN) (Fernandez-Corcuera et al. 2013a; Sheline et al. 2009), a network of medial brain regions that includes the hippocampus, medial PFC and inferior parietal cortex and is implicated in self-referential thoughts (Raichle et al. 2001). Unaffected first-degree relatives of patients with mood disorders display similar abnormal activity in the PFC (Allin et al. 2010; Callicott et al. 2003; Drapier et al. 2008). Aberrant task-related activity in dIPFC and mPFC thus seems to be among the most sensitive and reproducible neural markers of cognitive dysfunction across neuropsychiatric and genetically predisposed individuals. Given the involvement of dIPFC and mPFC in strategic encoding and selection and maintenance of information in working memory, these functional prefrontal deficits are thought to reflect problems with tactical memory encoding and with active working memory processes (Ragland et al. 2009).

Addressing points (ii) and (iii), the review of candidate cognition treatments in mood disorders in chapter II revealed EPO as the only compound to date with beneficial (albeit preliminary) effects on cognition across UD and BD. The recent line of EPO research led by the author exemplifies this stepwise approach in treatment discovery targeting cognition addressing points (i)-(iii) and may therefore provide important clues to a putative neurocircuitry-based biomarker for cognitive enhancement.
III. Why is erythropoietin a promising cognition treatment?

This chapter is largely based on paper II.

A. Neuroplasticity, cognition and erythropoietin

The multifunctional trophic growth factor EPO may represent a unique therapeutic agent to target deficits in neuroplasticity and cognition in mood disorders. Erythropoietin is a body-own protein produced predominantly in the kidney that was originally identified for its role in erythropoiesis and has been used for several decades in the treatment of anemia and illicitly for blood doping in competitive sports. Until recently, it was the general assumption that EPO improved athletic performance through its upregulation of red blood cells which leads to increased oxygen delivery to the muscles. However, intriguing new evidence indicates that the mechanism by which EPO increases physical endurance may be unrelated to blood oxygenation (Annaheim et al. 2016; Schuler et al. 2012). Indeed, the biological function of EPO is not confined to the hematopoietic system but involves multiple actions on tissues and organs throughout the body including the brain (Marti et al. 1996). Erythropoietin and a specific EPO receptor (EPO-R) system are present in the CNS in rodents, monkeys and humans and are important for in neurodevelopment (Marti et al. 1996), neurogenesis (Tsai et al. 2006) and neuroprotection (for review see (Sargin et al. 2010)). Erythropoietin is produced by neurons and astrocytes and this brain-derived EPO is increased up to a 100-fold by hypoxia which serves as an endogenous neuroprotective mechanism (Sargin et al. 2010). Consistent with this, there is a high EPO and EPO-R expression in neurons within brain regions particularly susceptible to acute hypoxia such as the hippocampus (Digicaylioglu et al. 1995). Endogenous EPO/EPO-R also have beneficial effects on cognition, especially in CNS conditions marked by cognitive decline such as schizophrenia as demonstrated in a large gene association study (Kastner et al. 2012). Specifically, carriers of the EPO and EPO-R genotypes associated with high EPO expression display highly superior short-term memory and speed of processing (Kastner et al. 2012). In keeping with this, mice with transgenic expression of constitutively active EPO-R in hippocampal and cortical neurons show superior memory and processing speed (Kastner et al. 2012). Notably, depressed patients have been found to have elevated cerebrospinal fluid levels of EPO, which is hypothesized to represent an endogenous attempt at brain repair, and is normalized with antidepressant treatment (Nakamura et al. 1998). Consistent with a role of brain-derived EPO in repair mechanisms, electroconvulsive seizure induces robust EPO gene expression and increased hippocampal EPO in animal models of depression (Girgenti et al. 2009). This converging evidence points to the importance of endogenous EPO and EPO-R for neuroprotection, neuroplasticity and cognition.

A conceptual therapeutic breakthrough occurred with the discovery that systemically administered EPO enters the brain and has neuroprotective and neurotrophic effects in traumatic, hypoxic-ischemic, excitotoxic and inflammatory brain damage and in animal models of
neurodegenerative and neuropsychiatric conditions (for reviews see (Brines et al. 2000; Sargin et al. 2010)). It is still unclear whether EPO crosses the blood-brain barrier through an active transport mechanism or in an unspecific manner. However, from a therapeutic perspective it is evident that in high doses (>500 IU/kg), systemically administered EPO enters the brain to an extent that is sufficient for neuroprotection. Indeed, pioneering translational studies revealed that weekly high-dose (40,000-48,000 IU) EPO treatment for 8-12 weeks improves cognitive function in multiple sclerosis and schizophrenia (Ehrenreich et al. 2007a; Ehrenreich et al. 2007b). Magnetic resonance imaging (MRI) of patients in the schizophrenia study showed that the EPO-associated improvement of cognition was accompanied by prevention of grey matter loss across regions that are typically affected in schizophrenia, including the hippocampus and fronto-parietal regions (Wustenberg et al. 2011). In contrast, low-dose EPO (8,000 IU; i.e., <500 IU/kg) produced no cognitive benefits (Ehrenreich et al. 2007a), indicating that high doses of EPO are required to achieve neuroprotection.

Multiple neurobiological mechanisms may underlie the beneficial effects of EPO on cognition. These include activation of anti-apoptotic, anti-oxidant and anti-inflammatory signaling in neurons, glial and cerebrovascular endothelial cells, and promotion of dendritic sprouting, neurogenesis, hippocampal brain-derived neurotrophic factor (BDNF) and long-term potentiation (Byts and Siren, 2009; Girgenti et al. 2009; Leconte et al. 2011). Erythropoietin was also shown to exert neuroprotective effects by inhibiting the activity of the enzyme glycogen synthase kinase 3 beta (GSK3β) (Ge et al. 2012; Li et al. 2015). This may be particularly relevant in relation to mood disorders given evidence that GSK3β is a key activator of cell death and is involved in mood disorders, hippocampal volume, glucocorticoid regulation, and neuroplasticity (Inkster et al. 2009). The cognitive effects of EPO thus seem to be mediated through multiple mechanisms which alone or in orchestra contribute to restoration of normal neuronal function and plasticity in conditions of neural damage or degeneration.

B. Preclinical and human fMRI studies of erythropoietin
Based on this encouraging evidence from preclinical and clinical studies for pro-cognitive effects, we conducted a systematic review of extant evidence for EPO as a candidate treatment for cognition and mood symptoms (paper II). We identified two preclinical studies of the effects of EPO on hippocampus-dependent learning and memory (Adamcio et al. 2008; Mogensen et al. 2004) and three human proof-of-concept fMRI studies of the effects of EPO on memory and neurocircuitry activity in healthy and depressed individuals (Miskowiak et al. 2007a; Miskowiak et al. 2007b; Miskowiak et al. 2009). The first published preclinical study of the effects of EPO on hippocampus-dependent memory by Mogensen, Miskowiak and colleagues (2004) demonstrated that a single dose of EPO vs. saline improves functional recovery after fimbria-fornix transection, which impedes all in and output of the hippocampus, in rats. No memory improvement was observed in intact (sham-operated) rats after this single dose of EPO, although
interestingly these rats displayed less stress-related behaviour after EPO treatment (Mogensen et al. 2004). Another study of repeated EPO-treatment of healthy mice every other day over three weeks revealed enduring EPO-associated improvement of hippocampus-related (contextual) memory (Adamcio et al. 2008). Repeated EPO doses may thus be required to achieve cognitive improvement in healthy animals. Interestingly, this memory improvement lasted for at least three weeks after treatment completion (Adamcio et al. 2008) at which time point haematocrit levels had normalised in the EPO-treated mice, indicating that the memory and haematopoietic effects of EPO are not directly related. Indeed, in-vitro analysis demonstrated that the beneficial effect on hippocampus-dependent memory function was associated with enhanced synaptic plasticity and long-term potentiation (LTP) in the hippocampus (Adamcio et al. 2008). Another study involving EPO administration twice weekly over 5-6 weeks to healthy mice also showed EPO-related improvement of hippocampus-dependent memory (Leconte et al. 2011). These effects are interesting in light of the persistent deficits in hippocampus-dependent memory in patients with mood disorders. Notably, the effects seem unrelated to the haematological changes since similar hippocampus-dependent memory improvement was also demonstrated with a non-erythropoietic variant of EPO, carbamylated EPO (CEPO) (Leconte et al. 2011). Together, these preclinical studies highlight EPO as a strong candidate treatment for memory deficits in mood disorders.

The first proof-of-concept fMRI studies of EPO in humans were conducted by the author and colleagues and investigated the effects of a single high dose of EPO (40,000 IU; similar to previously used doses (Ehrenreich et al. 2007a; Ehrenreich et al. 2007b) vs. placebo to healthy volunteers on hippocampus-dependent memory one week after administration (Miskowiak et al. 2007b). The one week interval between EPO administration and testing was chosen because of a similar time-lag for downstream effects of neurotrophic signalling to emerge and for newborn neurons to develop dendrites and begin their functional integration into the hippocampal circuit (Aimone et al. 2006). Functional effects of EPO-induced neuroplasticity were hence hypothesised to emerge with a delay of several days after its administration. The study revealed enhanced memory-related hippocampus response following EPO vs. placebo administration in the absence of changes in red cell mass (Miskowiak et al. 2007b). Greater hippocampal response in EPO-treated volunteers is consistent with improved recognition memory (Cansino et al. 2002) and enhanced hippocampal plasticity and neurogenesis one week after EPO administration (Ransome and Turnley, 2007). In contrast, we found no effect of EPO on memory-relevant hippocampal response 3 days after administration (Miskowiak et al. 2007a). This supports the hypothesis that the effect after one week originated from increased neurotrophic signalling given the time-lag of several days for functional effects of increased neuroplasticity to emerge (Aimone et al. 2006).

Based on these encouraging effects of EPO on memory-relevant hippocampus activity in healthy volunteers we investigated the effects of EPO vs. placebo in a clinically depressed population three days after administration (Miskowiak et al. 2009). Using an emotional picture encoding
paradigm revealed that EPO increased neural response in the left hippocampus and in right-side fronto-parietal regions during encoding of positive compared with negative pictures in depressed patients (Miskowiak et al. 2009). This effect of EPO is opposite to the hippocampal over-recruitment during encoding of negative pictures, which may be a neural mechanism of the negative memory bias in depression (Hamilton and Gotlib, 2008). The increased fronto-parietal response during encoding of positive vs. negative pictures following EPO administration is thus consistent with stronger encoding of positive information (Pourtois et al. 2006). Importantly, these effects on neuronal responses were accompanied by greater memory specificity in EPO-treated patients. Erythropoietin thus seems to improve some aspects of memory in depressed individuals, an effect that could be clinically important given the pervasive memory deficits in this patient group.

In conclusion, the findings of the reviewed studies in paper II highlight EPO as a candidate agent for neuroprotective add-on treatment strategies in mood disorders to improve cognitive function. Specifically, modulation of task-related neural activity in the PFC and temporo-parietal regions is a promising candidate biomarker model for cognitive enhancement given the aberrant activity in this neural network in mood disorders and the EPO-associated prefrontal and temporo-parietal ‘target engagement’.
IV. What can we learn from the erythropoietin trials in mood disorders?

This chapter is based on papers III-X.

A. Can erythropoietin reverse cognitive deficits?

Encouraged by the evidence for EPO-associated improvement of cognition in preclinical studies and neurocircuitry target engagement in the human proof-of-concept fMRI studies we set up two parallel clinical trials to investigate the potential of EPO to treat cognitive dysfunction and mood symptoms in treatment-resistant depression (TRD) and BD (papers III, IV and V). At the time of the EPO study start in 2009, the field was at an early stage with only a handful of previously published RCTs targeting cognition in BD and about a dozen studies in UD (see paper I and (Bortolato et al. 2016)). The EPO trials were the first to examine whether targeting deficits in neuroplasticity with repeated administration of high-dose EPO translates into beneficial effects of cognitive function and mood symptoms in mood disorders. The trials included 40 patients with TRD in a moderate depressive state and 44 partially remitted BD patients with persistent subjective cognitive difficulties. Patients were randomised to eight weekly EPO (40,000 IU) or placebo (saline) infusions in a randomised, double-blind, parallel-group design. They were assessed at with neuropsychological tests in weeks 1 (baseline), 9 (one week after treatment completion) and 14 (six weeks follow-up) and underwent MRI scanning in weeks 1 and 14. Mood symptoms, subjective cognitive function and quality of life were evaluated in weeks 1, 5, 9 and 14 (see Figure 1). For details on screening procedures and safety precautions, see paper III.

Figure 1. Erythropoietin study design and flow.

All patients with TRD showed substantial mood improvement from baseline to week 9 (primary outcome assessment time). Erythropoietin had no effect on the primary measure of depression severity, the Hamilton Depression Rating Scale 17-items (HDRS-17) score although some
additional depression-relevant read-outs including self-rated depression and quality of life showed improvement in EPO vs. placebo groups (paper IV). The most remarkable finding was that EPO-treated patients displayed pronounced, long-lasting improvement of verbal learning and memory that was twice the size of our a priori estimation of a clinically relevant change (see papers III and IV). In fact, comparison with norms from an age-matched healthy population showed that EPO improved memory from below to above the level of healthy individuals (see Figure 2).

**Figure 2.** Percent improvement from individual baseline in a verbal memory composite score obtained with the Rey Auditory Verbal Learning Test (RAVLT). This was obtained by summation of the four z-transformed RAVLT measures, addition of 10 to these summed score to obtain overall positive values and calculation of percent change from individual baseline. P-values indicate the results of the ANCOVA of the mean change from the individual baseline between the drug groups. The dotted line denotes the estimated RAVLT memory composite of healthy, age-matched individuals of average intelligence (Strauss et al. 200). Mean and standard error of the mean are presented. The original figure can be found in Miskowiak et al, Journal of Clinical Psychiatry, 2014 (paper IV). Copyright 2014. Physicians Postgraduate Press, Reprinted with Permission.

We did not re-examine memory beyond six weeks after treatment completion; however, it is conceivable that the effects could have persisted longer term given the previous demonstration of sustained cognitive effects of a similar EPO treatment-regime in patients with multiple sclerosis for up to six months (Ehrenreich et al. 2007a). The effect is noteworthy since memory may be the aspect of cognitive function with highest ecological validity (i.e., closest association with functional capacity) (Chaytor and Schmitter-Edgecombe, 2003) and seems particularly important for patients’ occupational function (Bonnin et al. 2014).

The study in remitted BD patients revealed only a trend toward EPO-associated verbal memory improvement (primary outcome) (paper V). Posthoc data analysis suggested that this could be partially explained by an absence of objectively-measured verbal memory impairments at baseline despite patients’ subjective complaints. However, we found a substantial EPO-associated improvement in sustained attention, social cognition, processing speed and executive function (secondary and tertiary cognition measures) in comparison with placebo. In particular, the effect on sustained attention (secondary outcome) would have survived Bonferroni correction for multiple comparisons across the two secondary and all primary, secondary and tertiary cognition
measures. Further, exploratory analysis of an overall ‘speed in complex cognitive processing’ composite score, ranging from attention to learning and executive function revealed a highly significant, long-lasting improvement in EPO versus saline groups (Figure 3).

**Figure 3.** Percent improvement from individual baseline in the cognition composite score of ‘overall speed of complex cognitive processing. This was obtained by: 1) summation of the z-transformed scores from tests probing the different cognitive domains: RAVLT total recall (verbal memory), Rapid Visual Processing speed for correct responses and RBANS coding (attention), WAIS letter-number sequencing, Trail Making B, and verbal fluency letter D (executive function), 2) addition of 15 to these summed scores to obtain overall positive values, and 3) calculation of percent change from individual baseline. Error bars denote standard errors of the mean. P-values denote the results of the ANCOVA of the raw composite scores. The dotted line denotes the estimated mean cognitive composite score of healthy, age-matched individuals calculated by z-transformation and summation of the average norms for healthy individuals on these tests. The original figure can be found in Miskowiak et al, Neuropsychopharmacology, 2014 (paper V). Reprinted with permission.

Notably, secondary analysis showed that the EPO-associated improvement of speed of complex cognitive processing occurred across both BD and TRD groups and persisted until at least six weeks after treatment completion (paper X). This observation corroborates with EPO-associated improvement in similar complex cognitive processing speed in multiple sclerosis and in schizophrenia (Ehrenreich et al. 2007a; Ehrenreich et al. 2007b) and suggests that broad pro-cognitive effects of EPOs occur across several neuropsychiatric patient groups with cognitive deficits. Notably, the EPO-associated improvement in speed of complex cognitive processing correlated moderately with reduction in subjective cognitive complaints. Multiple linear regression analyses with adjustment for clinical characteristics showed that in the acute treatment phase (weeks 1-9) this correlation was mediated by change in depressive symptoms. However, at the six-week follow-up assessment after treatment completion, the association between improvement in objective and subjective cognition was direct (i.e., not mediated by change in mood symptoms or any other variables). An analogue to this time-lag between the EPO-associated improvement in objective and perceived cognitive function is the typical delay between the physical healing of a sprained ankle and a person’s resumption of habitual daily activity levels. It is thus likely that it takes time for objective cognitive improvement to translate into better cognitive functioning in daily life. Notably, we found no correlation between EPO-
associated improvement in cognitive function and changes in quality of life or self-reported work capacity. A possible explanation is the short (six weeks) time interval between EPO treatment completion and the follow-up assessment. Longer follow-up times (e.g. six months) may be necessary to capture treatment-associated improvement in socio-occupational functioning. Alternatively, the EPO-associated objective cognitive change may have been insufficient to improve vocational function and life quality in this cohort with great illness chronicity. In keeping with this interpretation, Bonnin and colleagues (2016) demonstrated in a posthoc analysis of cognitive change in cognitively impaired patients from their functional remediation trial (Torrent et al. 2013) that treatment-related verbal memory improvement did not correlate with socio-occupational improvement (Bonnin et al. 2015). Taken together, these findings indicate that the relation between improvement in cognition and functional capacity is complex and possibly mediated by several additional factors, including residual depression and mania symptoms, illness chronicity and cognitive reserve (Anaya et al. 2016; Bonnin et al. 2015; Mur et al. 2009; Tabares-Seisdedos et al. 2008).

EPO-treated patients showed concomitant up-regulation of haematocrit during the acute treatment phase and we therefore cannot exclude the possibility that the observed effects of EPO on cognition were in part mediated by indirect haematological actions and brain oxygenation. However, several factors speak against this. Studies in other patient populations were able to dissociate between the effects of EPO on cognition and on haematological parameters (Ehrenreich et al. 2007a; Ehrenreich et al. 2007b; Miskowiak et al. 2007b; Miskowiak et al. 2008; Miskowiak et al. 2009). In keeping with this, we found no correlation between the changes in haematocrit and in cognitive performance from baseline to week 9 and the cognitive effects persisted for at least one month after normalisation of red blood cells (papers IV and V). Further, the absence of differential change in depression or mania symptoms in response to EPO vs. saline and the adjustment for mood symptoms in analyses of all cognition measures suggest that the improvements in cognition were mediated through a mechanism beyond that of symptom reduction. Indeed, we had observed in our early proof-of-concept fMRI studies that EPO produced neurocircuitry target engagement and improves some aspects of cognition in healthy and depressed individuals without affective red blood cells or mood (see paper II). Together, these findings point to a central CNS mechanism such as modulation of neuroplasticity.

In conclusion, the EPO trials in mood disorders are the first of their kind and provided promising - albeit still preliminary - evidence for beneficial effects of EPO across several aspects of cognition in patients with mood disorders. The beneficial effects of EPO on objective measures of cognition were accompanied by subjective cognitive improvement, indicating a clinical relevance of these effects. Based on these findings, larger clinical studies of EPO as a treatment for cognitive deficits in mood disorders are mandatory. Further, insight into the neuronal underpinnings of the EPO-associated improvement in cognition is pivotal for understanding the brain mechanisms of these effects.
B. What are the neuronal correlates of erythropoietin-associated cognitive improvement?

As EPO-associated cognitive improvement and underlying neuronal changes were not expected to be disease specific (Ehrenreich et al. 2007a; Ehrenreich et al. 2007b; Miskowiak et al. 2007a; Miskowiak et al. 2014a; Miskowiak et al. 2014b; Miskowiak et al. 2015b), we pooled neuroimaging data from the two EPO trials to increase the statistical power for detection of treatment-related structural and functional neurocircuitry change (Miskowiak et al. 2014a; Miskowiak et al. 2014b) (papers VI–VIII). Erythropoietin-related functional changes in memory- and working memory-relevant neural circuitries were investigated with a picture encoding and a spatial n-back working memory (WM) paradigm, respectively. In the picture encoding task, patients viewed a series of pictures and were instructed to indicate whether the scenes were indoors or outdoors and to memorize the pictures. This was followed by a free recall task immediately after the scan. The n-back WM task consisted of 1-back, 2-back and 0-back (control) conditions, during which the patients indicated with a button press whenever a ball appeared in the same square of a grid as one or two trials back (low-load and high-load conditions, respectively) or in one of the four corners of the grid (0-back control condition).

Complete structural data from the baseline and follow-up assessments was available and analysed for 69 patients (EPO: N=35, saline: N=34). EPO treatment prevented brain matter loss in a subfield of the left hippocampus encompassing the CA1-3 and subiculum and improved verbal memory in comparison with saline treatment across the BD and TRD cohorts (paper VI; Figure 4). Posthoc multiple linear regression analyses revealed that the structural increase in this hippocampal subfield was the only significant mediator of verbal memory improvement whereas no significant influence was found of change in mood symptoms, diagnosis, age or gender. Unlike the previous demonstration of EPO-related volume increase across several brain regions in schizophrenia (Wustenberg et al. 2011), we observed no effects of EPO on total grey matter volume in the hippocampus or in cortical regions in patients with mood disorders. This discrepancy may be explained by preferential neuroprotective effects of EPO in areas marked by neural degeneration, which is more wide-spread in schizophrenia, or – alternatively - by the four weeks shorter treatment in the present trials.

Figure 4. A EPO reduces brain matter loss in a subfield of the left hippocampus corresponding to CA1-3 and subiculum, as reflected by expansion in mean vertex location in this subfield in EPO versus saline treated patients. B. Mean hippocampal surface displacement in millimeters (change in mean vertex location) in the left hippocampal CA1-3 and subiculum revealed expansion in the EPO-treated patients (p=0.001) and shrinkage in those given saline patients from baseline to week 14 (p = 0.008). C. Linear relation between subfield hippocampal volume change and change in verbal memory; there was a highly significant positive correlation across all patients (r(68) = 0.40, p=0.001) as well as in the EPO group (r(34)=0.46, p=0.005). The original figure can be found in Miskowiak et al, Biological Psychiatry, 2015 (paper VI). Reprinted with permission.
**Functional MRI** data sets were available and analysed for 62 patients (EPO: N=32, saline: N=30) for the memory encoding task (paper VII) and for 56 patients (EPO: N=30, saline: N=26) for the spatial working memory task (paper VIII). Erythropoietin improved picture recall and increased encoding-related bilateral dIPFC and left-side temporo-parietal response (but not hippocampal activity) in comparison with saline (paper VII; Figure 5). Across the entire cohort, picture recall correlated positively with encoding-related activity in the dIPFC and temporo-parietal regions at baseline, and change in recall success correlated with activity change in these regions from baseline to follow-up.

**Figure 5. A.** Percent improvement in picture recall from individual baseline. This was calculated as the improvement from baseline to follow-up adjusting to each patient’s individual starting point. Error bars denote the standard error of the mean. Erythropoietin-treated patients showed greater memory improvement than those given saline from baseline to follow-up. **B.** Lower part. Neural response during picture encoding across all participants at baseline across the brain (green). Clusters show increased response in the erythropoietin (EPO) vs. saline groups in the exploratory whole-brain analysis (yellow, red, light blue and dark blue). Images are thresholded at Z>2.0 and P<0.05, corrected for multiple comparisons at a cluster level. **Upper part.** Plot of mean percent blood-oxygen-level-dependent (BOLD) signal change during encoding within these regions of interest in the EPO (red bars) and saline (blue bars) groups at baseline and follow-up. Bars show the mean; error bars show the standard error. Compared to saline, EPO increased BOLD signal change during picture encoding in these regions from baseline to follow-up. The figure is based on two original figures that can be found in Miskowiak et al, ACTA Psychiatrica Scandinavica, 2016 (paper VII). Reprinted with permission.
EPO also improved n-back working memory accuracy, which was accompanied by EPO-associated increase in the right dorsal PFC activity during high working memory loads and stronger deactivation of the left hippocampus that was part of the DMN (paper VIII; Figure 6). Across all patients, there was a correlation between WM accuracy and WM-related prefrontal activity at baseline and between WM improvement and increase over time in WM-related prefrontal activity and hippocampal deactivation. Notably, the observed effects of EPO on BOLD response and cognitive performance were not associated with changes in mood, hemoglobin or blood pressure.

Figure 6. A. Neural network activated during high WM load (2-back>1-back) across all patients at baseline (green); Region in the superior frontal gyrus (SFG) showing increased response specifically during this high WM load in the erythropoietin (EPO) group in the exploratory whole-brain analysis (yellow); A priori ROI in the right dorsolateral prefrontal cortex (dIPFC) (red). B. Plot of change from baseline to follow-up in mean % BOLD signal change during high WM load (2-back>1-back) within SFG and dIPFC in EPO (red bars) and saline (blue bars) groups at baseline and follow-up. At the post-treatment fMRI, EPO was also associated with greater activity in the dIPFC relative to saline. C. Default mode network (DMN) showing deactivation during WM (i.e., regions showing a negative linear relation with increasing WM load) across all participants at baseline (green); The (overlapping) functional cluster within the left hippocampus identified as showing WM-associated hippocampal deactivation (blue). D. Plot of mean % blood oxygen level dependent (BOLD) signal change during high-load WM (2-back>0-back) within the left hippocampal ROI in the erythropoietin (EPO) (red bars) and saline (blue bars) groups at follow-up. Images are thresholded at Z>2.0 and p<0.05, corrected for multiple comparisons at a cluster level. Bars show the mean; error bars show the standard error. Erythropoietin produced greater WM-related deactivation compared with saline at follow-up. E. Accuracy of n-back working memory reflected by arcsine transformed d' values in EPO and saline treated patients. Erythropoietin improved WM accuracy in comparison with saline from baseline to follow-up. The figure is adapted from figures 1 and 2 in K. W. Miskowiak, M. Vinberg, L. Glerup, O. B. Paulson, G. M. Knudsen, H. Ehrenreich, C. J. Harmer, L. V. Kessing, H. R. Siebner and J. Macoveanu, (2016), “Neural correlates of improved executive function following erythropoietin treatment in mood disorders”, Psychological Medicine, volume 46, pp 1679-1691, Cambridge University Press (paper VIII). Reprinted with permission.

Whereas the EPO-associated increase in dIPFC activity during picture encoding was in line with our hypothesis, the absence of functional hippocampal correlates of the structural increase within the left hippocampal CA1-3 and subiculum (Miskowiak et al. 2015b) was unexpected. A potential explanation is that hippocampal activity during picture encoding may not be a sensitive assay of the functions performed by the left hippocampus. Indeed, there is some evidence for specialization of the hippocampi, with the left hippocampus being preferentially activated during
verbal memory retrieval and the right hippocampus being more engaged in pictorial memory processes (Papanicolaou et al. 2002). This could explain why the EPO-associated left hippocampal volume increase correlated with verbal memory improvement but not with hippocampal activity during picture encoding. The absence of EPO-associated effects on hippocampal activity also contrasts with the enhanced bilateral hippocampal response during picture retrieval after a single EPO administration to healthy volunteers (Miskowiak et al. 2007b). This discrepancy may be related to differences in the implemented memory paradigms (picture encoding vs. retrieval) or study populations (healthy volunteers vs. patients with mood disorders). Alternatively, the neural mechanisms of EPO treatment may change over time, with early enhancement of hippocampal encoding, which translates into stronger strategic top-down mechanisms and improved memory performance after long-term treatment.

The effects of EPO on task-related neuronal activity may counteract the dorsal prefrontal hypoactivity during memory encoding (Dietsche et al. 2014; Hall et al. 2010; Kelley et al. 2013) as well as prefrontal hypo-activity and failure to suppress DMN activity during WM performance (Fernandez-Corcuera et al. 2013b; Garrett et al. 2011; Pomarol-Clotet et al. 2015; Sheline et al. 2009; Siegle et al. 2007) in mood disorders. Notably, explicit memory training produces similar increases encoding-related activity in the medial and lateral PFC and in temporo-parietal regions across healthy individuals and patients with mild cognitive impairment (Hampstead et al. 2011; Kondo et al. 2005). The effects of EPO on dIPFC and temporo-parietal responses may thus reflect strengthened visuospatial mnemonic processes, consistent with the general correlation between the increase in neural activity within these regions and recall success. Such neural effects of EPO may occur early in treatment, as demonstrated by increased prefrontal and temporo-parietal activity during picture encoding one week after a single dose of EPO to healthy volunteers (Miskowiak et al. 2007b).

During the WM task, the greater suppression of hippocampal activity in the EPO group may indicate better disengagement from task-irrelevant thoughts, consistent with the observed correlation between suppression of hippocampal activity and higher WM accuracy. The PFC shows low activity levels at low WM load, increased activity at high load, and decreased activity when the load exceeds WM capacity (Callicott et al. 1999). Given this, the greater prefrontal response specifically during high WM loads in EPO vs. saline treated patients at follow-up suggests that EPO may prevent break-down of WM capacity. These results are in accordance with increased dorsal PFC activity during executive function tests after cognitive training in multiple sclerosis (Filippi et al. 2012) and schizophrenia (Ramsay and MacDonald, III, 2015). The observed effects on neural activity may not be specific to EPO but also occur in response to other interventions with pro-cognitive effects. This would highlight normalisation of hypo-activity in dorsal PFC regions and DMN hyper-activity during strategic encoding and executive control tasks as key neurobiological targets associated with pro-cognitive effects of both biological and psychological treatments across a range of neuropsychiatric disorders.
For fMRI measures to become surrogate endpoints for clinical efficacy, the treatment-related changes in the putative neurocircuitry biomarker model must be linked to the clinical outcome – i.e., correlate with or predict a change in the disease phenomenon (Wong et al. 2009). Indeed, the observed EPO-related structural and functional brain changes correlated with observable changes in behavioural performance on memory and executive function, in support of these neuronal changes potentially representing surrogate endpoints of cognitive enhancement.

Several neurobiological mechanisms may underlie the structural hippocampal increase and the task-related activity changes in EPO versus saline-treated patients. Although EPO increased haematocrit during the active treatment phase, red blood cells were normalized at the time of the follow-up scan six weeks after treatment completion. While patients’ concomitant medication may have had unspecific effects on neural activity, there were no differences between EPO and saline groups in medication status and posthoc analyses showed no correlation between neural activity in the identified regions and medications. Blood pressure has been shown to correlate positively with fMRI BOLD response (Wang et al. 2006). However, we found no significant differences in blood pressure between groups or correlations between EPO-associated change in BOLD response and blood pressure. The visual control task also showed no difference between groups in occipital activation to photic stimuli, suggesting that the observed effects of EPO did not result from any global hemodynamic changes. Finally, there were no significant effects of EPO on mood symptoms across the UD and BD groups or correlations between changes in mood and in hippocampal volume, neural responses or task performance. There has been great recent media attention to the questionable validity of the most common fMRI software packages (i.e., SPM, AFNI and FSL ‘OLS option’), which may be associated with high familywise error rates (FWE; i.e., false positives) (Eklund et al. 2016). Importantly, the fMRI analyses in the EPO studies employed a full mixed-effects approach at the group level (FSL ‘FLAME1’) (Woolrich et al. 2004), which is a valid method with low FWE rates (<5%) (Eklund et al. 2016).

In conclusion, the neuroimaging studies (papers VI-VIII) provide a cerebral basis for the EPO-associated improvement of cognitive function in patients with mood disorders. The findings are consistent with the ability of EPO to enhance neuroplasticity and cognition in animal studies and with the early proof-of-concept evidence for EPO-related target engagement in hippocampus, PFC and related neural circuitries. Together, the findings highlight EPO as a candidate treatment to target deficits in neuroplasticity and cognition in mood disorders. Beyond EPO, the findings provide the first evidence for a potential circuitry-based biomarker model for pro-cognitive effects.

C. Which patients showed greatest cognitive benefits of erythropoietin?

Insight into how we can target cognition treatments to patients with mood disorders is urgently needed, as we saw in the systematic review of cognition trials in BD (paper I). In particular,
post-interventional investigation of into what characterized the patients who showed greatest cognitive benefits of EPO may inform future screening strategies. Using such criteria in future screening of trial participants can ensure an enriched sample with good scope for cognitive improvement and hence increased statistical power for detection of treatment efficacy. A particularly critical issue that needs to be clarified is the role of baseline cognitive impairment. Indeed, a lesson learnt from the EPO trial in BD was that we should have ensured that participants displayed objective deficits in verbal memory (the primary outcome), since the absence of substantial verbal memory deficits in this cohort could be a reason for the negative finding regarding this aspect of cognition. In line with this, it has been suggested that the failure to detect cognitive efficacy of pramipexole and of functional remediation could be that patients were not sufficiently cognitively impaired (Bonnin et al. 2015; Burdick et al. 2012).

Given the beneficial cognitive effects of EPO across UD and BD, these cohorts presented a unique opportunity for investigating the clinical predictors of treatment efficacy on cognition (papers IX and X). These analyses revealed that patients with objective cognitive dysfunction at baseline, defined as performance scores one standard deviation (SD) or more below the normative mean, were substantially more likely to achieve a ‘clinically relevant’ EPO-associated improvement in verbal memory (paper IX) and in speed of complex cognitive processing (paper X) than patients with relatively intact baseline cognition (i.e., performance within one SD on the normative mean) (see Figure 7, upper part). In contrast, there was no such impact of baseline cognitive impairment on the chances of achieving a clinically relevant cognitive improvement in the saline group (Figure 7, lower part), suggesting that the impact of baseline impairment was specific to pro-cognitive treatment rather than simple regression towards the mean with repeated testing.

**Figure 7.** Upper part: EPO group. Plots of change in memory performance for the EPO-treated patients with intact baseline memory (RAVLT total recall scores >43) and baseline memory dysfunction (RAVLT total recall scores ≤43). The odds of a successful EPO treatment were increased by a factor 291 (CI 95%: 3-31316, p=0.02) for patients with memory dysfunction compared to patients with normal memory at baseline. Lower part: saline group. Plots of change in memory performance for the saline-treated patients with intact baseline memory and with baseline memory dysfunction, respectively. In this group, there were no differences in the odds of achieving a clinically relevant memory improvement between those with normal memory and memory dysfunction at baseline. The original figure can be found in paper IX: Miskowiak et al. Targeting treatments to improve cognitive function in mood disorder: Suggestions from trials using erythropoietin. Journal of Clinical Psychiatry (in press). Copyright 2016. Physicians Postgraduate Press. Reprinted with permission.
This is the first convincing evidence in the field for a profound influence of cognitive impairment at baseline on the chances of achieving treatment efficacy on cognition. Notably, these analyses were adjusted for mood symptoms, diagnosis and various demographic variables, which did not impact on the chances of treatment efficacy. Nevertheless, other factors – including the nature of the particular treatment under investigation - may also affect the chances of finding pro-cognitive effects in cognition trials. For example, post-hoc analysis of our negative trial of cognitive remediation in BD also revealed no efficacy on cognition in the subgroup of patients with objective cognitive deficits at baseline (Demant et al. 2015b). Negative findings in cognition trials therefore cannot exclusively be explained by the absence of cognitive impairment in study participants.

The impact of self-reported cognitive difficulties at baseline were less consistent; while greater subjective cognitive difficulties at baseline were associated with a significant but small (53%) increase in the chances for treatment efficacy on memory (paper IX), no such association was observed for chances of achieving a clinically relevant improvement in speed of complex cognitive processing (paper X). This inconsistent and small impact of subjective cognitive difficulties at baseline suggests that objectively-measured cognitive impairment is a more robust predictor of treatment efficacy on cognition. Interestingly, longer illness duration was associated with a small but significant incremental increase in the chances of treatment efficacy on memory (16% increase for every year of illness; paper IX). This preliminary evidence for greater cognitive benefits in more chronic patients is consistent with the staging model of affective
illness, according to treatments should be targeted to a patient’s particular illness stage, with chronic stages requiring more intensive interventions (Vieta et al. 2011). In line with this notion, EPO treatment may be particularly beneficial to target cognitive and functional impairments in patients with a more chronic illness course.

In conclusion, the substantially greater cognitive benefits of EPO in patients with objectively-measured cognitive dysfunction at baseline suggest that neuropsychological screening is warranted to verify that patients with cognitive complaints also show measurable objective deficits before their inclusion in trials targeting cognition.
V. Which psychological assessments should we use to screen for cognitive impairment?

Notwithstanding the clear importance of screening for cognitive impairment in cognition trials, we have no good screening tools for cognitive dysfunction in mood disorders. In particular, currently available screening tools developed for dementia or schizophrenia are associated with ceiling effects in (the generally less impaired) patients with mood disorders. Further, it is still unclear if objective or subjective criteria should be used as demonstrated by the systematic review of BD cognition trials (paper I and chapter II). There is therefore a pressing need for studies that validate novel, feasible screening instruments optimized for patients with mood disorders and to clarify whether objective or subjective measures have the best sensitivity and specificity for cognitive impairment.

Good clinical tools to screen for cognitive impairment in mood disorders must be brief and simple to administer and include objective measures across a range of cognitive domains including attention, memory and executive function. The Screen for Cognitive Impairment in Psychiatry (SCIP) is a brief (<20 min) neuropsychological instrument that was recently developed to screen for cognitive dysfunction in psychotic and affective disorders (Purdon, 2005). Emerging evidence indicates good validity, reliability and sensitivity of the SCIP for detection of cognitive impairment in BD (Guilera et al. 2009; Purdon, 2005; Rojo et al. 2010) which warrants further investigation of the SCIP to screen for cognitive impairment in mood disorders. The Cognitive Complaints in Bipolar Disorder Rating Assessment (COBRA; (Rosa et al. 2013)) is a newly developed self-report measure that examines the cognitive difficulties in daily life situations in BD. The COBRA has shown some correlation with objective measures of memory and executive functions (Rosa et al. 2013) and therefore warrants further investigation as a new subjective screening instrument for cognitive impairment in mood disorders. We therefore conducted two parallel studies in 84 BD and 53 UD patients, respectively, and 103 healthy controls (papers IX and XII) to investigate (i) the validity and reliability of the SCIP and COBRA and their optimal cut-offs for detection of cognitive impairment in UD and BD, respectively, (ii) whether optimal screening involves objective, subjective or a combined objective-subjective measure of cognitive impairment, and (iii) the functional relevance of these cognition measures through an examination of their association with socio-occupational capacity.

The SCIP and the COBRA were translated into Danish with permission from the authors. These Danish versions of the SCIP (SCIP-D) and the COBRA showed high concurrent validity in both BD and UD, as indicated by high correlation with a full standardised neuropsychological test battery and an established self-report measure of cognitive impairment, respectively. In the BD cohort, the SCIP-D showed high sensitivity and specificity (84% and 87%, respectively) for cognitive impairment at an optimal cut-off score of <70 (paper XI; Figure 8.A). In contrast, the COBRA showed suboptimal sensitivity and specificity (68% and 74%, respectively) for objective
cognitive impairment in BD at an optimal cut-off of >14, and combining these thresholds in a combined SCIP-COBRA measure showed lower decision validity than SCIP-D alone (84% sensitivity and 68% specificity) for detection of cognitive dysfunction. In the UD cohort, the SCIP-D had high sensitivity and good specificity for objective cognitive dysfunction (83% and 79%, respectively) at the optimal cut-off score of <74 (paper XII; Figure 8.B). Similar to the observations in the BD study, the COBRA showed poorer sensitivity and specificity (65% and 68%, respectively) for objective cognitive dysfunction at an optimal cut-off of >13. However, in this cohort the combined SCIP-COBRA measure provided the highest sensitivity while maintaining an acceptable albeit somewhat lower specificity (91% and 70%, respectively) for objective cognitive dysfunction at the optimal cut-off (paper XII; Figure 8.C).


Comparison of the BD and UD cohorts indicated that the discrepancy between the findings in these groups may be due to greater cognitive complaints (and more mood symptoms) in the UD patients despite similar levels of objective cognitive performance (paper XII). In particular, more negative mood-congruent bias could explain the greater subjective complaints and thus higher sensitivity of the combined SCIP-COBRA measure in the UD cohort (paper XII). In clinical settings, screening instruments should arguably favour sensitivity at the expense of specificity (Cuesta et al. 2011), indicating that a combined measure of subjective and objective cognition may be feasible. In cognition trials, however, the use the SCIP alone (favouring specificity) may be preferable to ensure inclusion of an enriched sample with objective impairment and hence
substantially greater scope for cognitive improvement. Nevertheless, these patients should also experience some cognitive difficulties to ensure their motivation for taking part in the trial.

Comparison of patients’ performance on three alternate SCIP-D versions indicated that these were equivalent and can therefore be applied for longitudinal assessment of cognitive status to minimize learning effects with repeated testing (paper XII). Correlations between the SCIP-D and COBRA measures were generally poor in both BD and UD but both measures were associated with socio-occupational difficulties. Notably, the association between the COBRA and socio-occupational difficulties was strongest, which may be a result of the implemented socio-occupational measure, the Functional Assessment Short Test (FAST) (Rosa et al. 2007), being partially based on patients’ subjective evaluations. Future studies are therefore warranted to investigate whether objective performance-based assessments of socio-occupational function, such as the UCSD Performance-based Skills Assessment (UPSA) test (Patterson et al. 2001) and the Assessment of Motor and Process Skills (AMPS) (Fisher, 1993; Patterson et al. 2001) show stronger correlations with objective cognitive impairment. Notwithstanding, the present and previous demonstrations of an association between psychosocial difficulties and both subjective and (albeit to a lesser extent) objective cognitive impairment indicate a clinical relevance of assessing and monitoring cognition in mood disorders.

In conclusion, the studies highlight the SCIP-D and the COBRA as valid and feasible instruments to screen for objective and subjective cognitive impairments, respectively, in partially remitted BD and UD patients. The SCIP-D provided the optimal sensitivity and specificity for detection of objective cognitive impairment in BD. In UD, the SCIP-D provided best specificity, while a combined SCIP-COBRA score provided the best sensitivity for objective cognitive impairment. Based on these findings, it seems feasible to use of a short objective screening instrument such as the SCIP (potentially combined with the COBRA) in mood disorders to correctly identify patients with objective cognitive dysfunction.
VI. General discussion

A. Summary of challenges in the field
Cognitive dysfunction is a core illness dimension and is increasingly recognized as a treatment target in mood disorders. The field is still in its infancy and cognition trials in mood disorders face several serious methodological challenges that may explain their overall disappointing results. Most studies are limited by a high risk of bias and methodological issues due to the lack of adherence to the CONSORT criteria for conduct of clinical trials (Moher et al. 2012) and the absence of consensus or guidelines for cognition trials in mood disorders. In particular, there is no agreement on the need to screen for cognitive impairment despite evidence for less severe cognitive deficits in mood disorders than in schizophrenia and a large group of cognitively intact patients. The lack of systematic screening for cognitive impairment is therefore a critical problem as potential inclusion of cognitively intact patients reduces the scope for treatment-associated improvement of cognition and, consequently, risk of type II errors. The problem is not easily solved, since it is also related to insufficient insights into the relation between subjective and objective cognition measures and hence whether we can simply rely on patients’ subjective cognitive difficulties or should implement objective neuropsychological tests to screen for cognitive impairment. A further complication is the lack of valid, short and feasible screening instruments for cognitive impairments in mood disorders. Given these challenges, screening for cognitive impairment has been implemented in only about one-fifth of trials in mood disorders, of which almost all used subjective criteria that have suboptimal sensitivity and specificity for cognitive dysfunction in mood disorders.

Another key problem is the lack of consensus or guidelines on how to monitor treatment efficacy on cognition. Specifically, most trials in the field have used multiple cognition outcomes without a priori hierarchy. For the cognition trials that did define a primary cognition outcome, there was no consistency in which outcome was chosen. Some trials also used subjectively experienced cognitive difficulties or socio-occupational function as the primary study outcome (e.g., (Deckersbach et al. 2010; Torrent et al. 2013)). Although this may ensure a clinical relevance of the primary outcome, the problem is that subjective cognitive difficulties and observer-based/self-reported socio-occupational function show only partial correlation with the objective cognitive deficits that the treatments aim to target. It therefore seems warranted to define the primary outcome as an objective measure of cognition and socio-occupational function as a key secondary outcome, which can elucidate the clinical relevance of potential objective cognitive improvement. The next challenge is the disagreement with respect to whether the objective cognition outcome should be a global composite score summarizing the performance across several domains or, in contrast, a specific cognitive domains/test measure. Given the critical importance of a sensitive, informative and agreed-upon primary outcome of cognition trials for the ability to claim ‘treatment efficacy’, this is a serious problem in the field. Other issues are
how to manage mood symptoms and concomitant medication since both influence cognitive function and may therefore confound the findings if not properly accounted for. Statistical analyses of efficacy outcomes are also highly variable across trials, and given substantial drop-out rates in many trials the lack of adjustment for missing data in the analyses is problematic.

Taken together, several methodological limitations can have contributed to the disappointing or only preliminary findings in the field, which has led several large pharmaceutical companies to desert this field because of too high risk and low returns. Given the profound impact that cognitive dysfunction in mood disorders on psychosocial functioning, work productivity and societal costs, it is imperative that we improve the methodology of cognition trials. The perhaps most fundamental impediment to success of treatment discovery programs is the lack of a neuroimaging biomarker model for cognitive enhancement and hence poor ability to predict efficacy of novel treatments. Given the lack of proven effective cognition treatments, the best starting point for identifying a valid neurocircuitry biomarker model is post-interventional fMRI assessment of the neuronal changes in response to the most promising candidate treatments in the field, such as EPO, which has provided novel evidence for a putative neurocircuitry-based biomarker for cognitive enhancement.

B. Contributions of the present research
The studies on which this dissertation is based suggested for the first time that the multifunctional growth factor EPO has mood-independent beneficial effects on cognition across UD and BD. Although the findings of the EPO trials can only be considered hypothesis-generating, they are among the strongest in the field given the general paucity of evidence for any beneficial cognition treatments in mood disorders. The EPO studies elucidated also for the first time the structural and functional neuronal changes within cognition-relevant networks that accompany treatment-related cognitive improvement in mood disorders. In addition, the secondary analyses of the trial data revealed that it was the patients with baseline deficits in objective (and to some extent also subjective) cognition who showed greatest cognitive benefits of EPO treatment. Based on this finding and the lack of feasible tools to screen for cognitive impairment in mood disorders, we conducted two studies in separate cohorts UD and BD patients which resulted in validation of feasible screening tools that can be implemented in future cognition trials.

The EPO trials taught us three lessons that have important implications beyond EPO, as they provide insight into how we can tackle the following major methodological challenges in the field: (i) the lack of consensus on the need to screen for cognitive impairment or with which assessment tools, (ii) the issue with selection of cognition outcomes for tracking treatment efficacy, and (iii) the lack a neurocircuitry-based biomarker model for cognitive improvement.

(i) First, the EPO trials demonstrated convincingly that there is a need for objective screening for cognitive dysfunction to verify that patients with cognitive complaints display measurable
deficits. Specifically, post-interventional analyses revealed that on average, BD patients showed no verbal memory deficits in comparison with norms of healthy, age-matched individuals despite their subjective cognitive complaints. This is a potential explanation for the lack of significant benefits of EPO on verbal memory (primary outcome) in the BD group alone, although the effect on memory rendered significant in the pooled analyses of the TRD and BD cohorts with greater statistical power. In support of this association between baseline impairment and scope for improvement, we observed that patients with verbal memory performance ≥1 SD below normal mean at baseline showed dramatically greater chances for treatment success on memory. Similarly, participants with performance ≥1 SD below normal on ≥2 tests at baseline displayed substantially greater chances of EPO treatment success in global cognition. It thus seems warranted in future cognition trials to use objective criteria for cognitive dysfunction within the targeted domains with screening tools such as the SCIP, assuming that the findings can be generalized to other cognition treatments. This may ensure ‘enriched’ cohorts, greater statistical power and thereby improved chances of success in future cognition trials.

(ii) It seems feasible to select a broad composite score rather than a specific cognitive test/domain as primary efficacy outcome. We had pre-specified verbal memory over sustained attention as primary outcome in the BD trial (Miskowiak et al. 2014a). Nevertheless, this choice was somewhat arbitrary; at the time of hypothesis generation there was equal evidence for verbal memory and sustained attention deficits in BD, and EPO had been found to improve both cognitive domains (Miskowiak et al. 2010b). Despite the negative primary outcome, the pronounced effect of EPO on the sustained attention (secondary outcome) was therefore considered encouraging. Indeed, it is recommended that priority between primary and secondary outcomes should be regarded with some degree of flexibility to allow for consideration of additional findings if these are substantial enough (38). Nevertheless, post-hoc analyses showed that a broader cognition measure would have been a more informative and sensitive primary outcome for detection of treatment efficacy that also revealed significant improvement across both the BD and TRD cohorts (papers V and X). Notably, such global composite score should include higher-order neuropsychological tests that are sensitive to the less severe cognitive deficits in mood disorders (i.e., avoiding ceiling effects) and are relatively unaffected by mood symptoms. Given some heterogeneity of cognitive deficits in mood disorders, a global composite score may also—by summarizing the changes across several domains—be a more robust measure than a single cognition test by picking up small cumulative treatment effects across several cognitive domains (Burdick et al. 2015). An alternative strategy could be to operationalize ‘treatment success’ by a particular number of tests that show clinically relevant improvement, such as treatment-related performance increase of ≥1 SD in at least two of six cognitive tests comprising the cognitive composite score as in our secondary EPO analysis (paper X). Either strategy could help resolve the problem with predicting which single cognitive domains are targeted by novel candidate treatments based on their pharmacological profile and effects in animal models. A key secondary outcome (perhaps together with socio-occupational function)
could then be a single higher-order cognition test with particular sensitivity to discrete cognitive impairment in mood disorders, such as a test of sustained attention (Jensen et al. 2016; Miskowiak et al. 2014a).

(iii) The third lesson from the EPO trials was that fMRI to examine early target engagement within the key neurocircuitry underlying the targeted cognitive domains (e.g. memory and executive function) can provide key proof-of-concept evidence regarding whether the intervention is likely to produce clinically relevant improvement of cognition in mood disorders and should therefore be further investigated. Specifically, we had first examined with proof-of-concept fMRI studies whether a single high dose of EPO vs. saline produced target engagement in memory- and working-memory relevant neural circuits across healthy and depressed individuals (Miskowiak et al. 2007b; Miskowiak et al. 2008; Miskowiak et al. 2009). The encouraging proof-of-concept evidence was the basis for our subsequent long-term clinical efficacy trials (papers IV and V). Remarkably, the observed EPO-related activity changes in dorsal PFC and temporo-parietal regions during strategic encoding and working memory in the eight-week trial were strikingly similar to the effects of a single dose of EPO vs. saline on neural activity in healthy volunteers (Miskowiak et al. 2008) and to the effects observed after two weeks intervention with vortioxetine in partially remitted depressed patients and healthy volunteers (Smith et al. 2016). In fact, the dIPFC target engagement could perhaps represent an even more general biomarker for pro-cognitive effects of both pharmacological and psychological interventions; A recent meta-analysis of the neural correlates for cognitive remediation in schizophrenia revealed increase in dorsal prefrontal activity as the most reliable marker of cognitive improvement in this patient group (Ramsay and MacDonald, III, 2015). Together, these findings from the early proof-of-concept and clinical efficacy trials provide putative neurobiological targets, which can be used in future cognition trials to examine early neurocircuitry target engagement with novel candidate cognition treatments.

C. Strengths and limitations of the present research

1. The erythropoietin trials
The EPO trial in BD was among the very few RCTs in the field with an overall ‘low’ risk of bias, as assessed with the Cochrane Risk of Bias Tool (paper I). Key reasons were the adequate randomisation and blinding of the trial, a priori priority between the cognition outcomes, a low dropout rate, and appropriate statistical analyses to adjust for missing data. It was a strength of the EPO trials that they were founded on proof-of-concept evidence for neurocircuitry target engagement from single-dose fMRI studies in healthy and depressed individuals. Pooling neuroimaging data from the two identical RCTs in TRD and BD ensured large sample sizes (N=56-69; i.e., 26-35 per group) and thus good statistical power. In comparison, structural MRI studies of mood disorders often involve 20-40 patients (and an equal number of healthy controls) in cross-sectional designs (Elvsashagen et al. 2013; Huang et al. 2013), and 10-30
patients in longitudinal designs (Frodl et al. 2008; Yucel et al. 2007), while prospective functional MRI investigations of cognition treatments in other brain disorders involved only 12-31 participants (6-16 per group) (Filippi et al. 2012; Subramaniam et al. 2014). Further, our assessment of cognitive performance in parallel with the neuroimaging measures enabled insight into the functional implications of the EPO-associated neuronal changes. A strength was also the randomised, placebo-controlled design, which accommodated for effects of repeated scanning and learning. Finally, the patient cohort was uniquely suited for investigation of baseline predictors of treatment efficacy on cognition given the observed beneficial cognitive effects of EPO across TRD and BD.

A key limitation of the EPO studies was that the evidence for efficacy of EPO on cognition is still preliminary, since improvements were seen only across the secondary and tertiary cognition outcomes. Nevertheless, the pronounced effect of EPO on the sustained attention in BD (secondary cognition outcome) and in the exploratory composite of ‘speed of complex cognitive processing’ across attention, memory and executive function across both diagnostic groups were encouraging. We did not correct p-values for multiple testing since such statistical methods for controlling p-values have limited value in clinical trials with pre-specified priority of outcomes (Pocock, 1997). However, if we had performed Bonferroni corrections the significant effect of EPO on sustained attention would have prevailed and persisted at the six weeks follow-up. Another limitation was the only modest sample sizes of the primary efficacy studies in TRD and BD (N=39 and N=43, respectively) which may have introduced type II errors. Nevertheless, we detected EPO-associated improvements across multiple cognition outcomes in both TRD and BD.

In comparison, other RCTs targeting cognition in BD included similar or smaller samples, with three studies including ≤20 participants, 12 studies including 24-68 participants and only one multicentre trial (in which cognition was secondary outcome) had a large sample with 77-82 participants per group (see paper I). Cognitive impairment was an inclusion criterion in the BD trial but this was assessed only subjectively, and we have subsequently found a poor correlation between subjective and objective cognition measures (see paper I). Indeed, post-hoc analyses revealed no general impairment in verbal memory in the BD patients which may have masked a potential effect of EPO on this primary outcome measure. Further, the extensive exclusion criteria in the trials may limit the generalizability of our findings to clinical practice where a substantial proportion of patients smoke, are over-weight or have significant medical comorbidities. However, the criteria were inevitable to ensure patient safety in these first trials of their kind, which was of principal importance. The EPO-associated increase in red blood cell levels during the active treatment phase could confound the interpretation of the effects of EPO as neural in origin. However, converging evidence from preclinical studies and our human proof-of-concept studies suggest that the ability of EPO to improve cognitive function is mediated by actions on neuroplasticity (see paper II). In keeping with this, the beneficial effects of EPO on cognition persisted for at least one month after red blood cell normalisation and were accompanied by structural and functional changes in cognition-relevant neural circuitries that
showed no correlations with change in haematological parameters. In the BD trial, it was also a limitation that there were more lithium-treated patients in the EPO versus saline groups since lithium may impair psychomotor speed and verbal memory in the acute treatment phase (Dias et al. 2012) and could have reduced the cognitive effects of EPO. However, post-hoc analyses revealed no interaction between the effects of EPO and lithium on cognition or differential treatment effects between patients with or without lithium treatment (Miskowiak et al. 2015a).

Pooling the data from the heterogeneous groups of TRD and BD patients with different symptom severity is a limitation, since these mood disorders may involve differential although partially overlapping pathogenic processes. Nevertheless, disruption of neuroplasticity, hippocampal volume reduction, cognitive deficits and aberrant cognition-related hippocampal and prefrontal responses occur across both disorders and EPO has been found to facilitate cognition and neuroprotection across a range of different brain disorders (Ehrenreich et al. 2007a; Ehrenreich et al. 2007b; Miskowiak et al. 2014a; Miskowiak et al. 2014b). Patients’ concomitant medication could have had non-specific effects on global brain activation. However, post-hoc analyses showed no evidence for such confounding effects: The EPO and saline groups were well-matched for medication status, and specific analyses of the potential influence of lithium showed no difference in EPO-associated improvement in patients with or without lithium or any interactions between EPO and lithium on the cognitive outcomes. Indeed, the effects of EPO vs. saline were evident over and above any non-specific effects of medication and prevailed after adjustment for medication status and dose.

There are no agreed-upon criteria for cognitive dysfunction in mood disorders. For the post-interventional analyses of who showed greatest cognitive benefits of EPO, our definitions of a ‘clinically relevant’ memory improvement of ≥6 points in RAVLT total recall (i.e., ≥4 points greater in EPO vs. saline groups) was somewhat arbitrary, although this was based on the definition from our originally published trial protocol (paper III). Nevertheless, verbal memory impairment has been consistently associated with functional disability in BD (Bonnin et al. 2010; Martinez-Aran et al. 2007), suggesting that improvement in this aspect of cognition may translate into increased functional capacity long-term. The definition of a clinically relevant cognitive improvement in overall ‘speed of complex cognitive processing’ was also somewhat arbitrary although partially informed by previous suggestions for cognition trials (Burdick et al. 2015). Another limitation was that the modest samples of EPO participants (N=40) for these post-interventional analyses of whom showed greatest treatment benefits. This resulted in wide confidence intervals of the estimated Odds Ratios for treatment efficacy of EPO on cognition and could have introduced type II errors. These analyses should therefore only be considered hypothesis-generating. Finally, the observed impact of baseline cognition were related solely to EPO treatment which may limit the generalizability of the findings, since the associations between baseline cognition and treatment success could be different with other cognition treatments.
2. The cognitive assessment studies

The primary strength of the cognitive assessment studies was that they were the first to validate new feasible screening tools for cognitive impairment across UD and BD patient cohorts. The large sample size of BD patients (n=84) was a strength, providing good statistical power for validation of the tools, determining optimal cut-off points, and investigating the associations between objective and subjective measures of cognition and socio-occupational function. In contrast, the modest sample of UD patients (n=53) may have resulted in type II errors. Another limitation is that patients were almost all on antidepressant or mood-stabilizing medications the might have accentuated (or attenuated) their cognitive dysfunction (Goldberg and Burdick, 2008). Nevertheless, post-hoc analyses showed that the results were relatively unaffected by medication status in accordance with meta-analytic findings (Bora et al. 2013; Bourne et al. 2013). Further, the studies were naturalistic, aiming to assess the validity of new screening instruments in partially remitted patients with mood disorders of whom the majority are medicated. Finally, the brevity of the SCIP –that ensures feasibility in the clinical setting for screening purposes– comes at the cost of more detailed insight into cognitive function. In particular, the SCIP includes no standard tests of executive function or problem solving ability. The SCIP therefore should not replace a full neuropsychological examination but be used only for screening purposes or for tracking treatment efficacy in severely ill patients for whom more extensive neuropsychological assessments are unfeasible.

D. Clinical limitations of erythropoietin and need for other treatments

Despite the promising evidence for EPO as a new add-on cognition treatment in mood disorders, four major limitations of EPO may impede its clinical use. The first problem is the hematopoietic action of EPO with repeated administration would necessitate close monitoring of haematocrit and thrombocyte levels and, potentially, blood lettings in these non-anaemic patient populations. Important next steps for further clinical development of EPO are therefore to investigate whether the unwanted hematopoietic activities of long-term EPO treatment can be avoided with more infrequent administration or with use of CEPO and other non-hematopoietic EPO analogues, if these reveal beneficial effects on cognition in humans. The second limitation is the concern that EPO could potentially promote malignant tumour growth although the evidence for this adverse effect remains controversial (Jelkmann et al. 2008). Low-dose EPO is widely used in the treatment of chemotherapy-induced anaemia to avoid red blood cell transfusions, and EPO is only avoided in non-anaemic cancer patients (Jelkmann et al. 2008). The third clinical limitation is the high costs associated with EPO treatment. Specifically, a 1 ml vial of EPO (40,000 IU) costs between 200 and 500 euros depending on the country. As enduring effects on cognition are likely to require repeated EPO administration over several weeks, this would impose great costs on patients and society. Ongoing research effort therefore aims to delineate the neurocognitive effects of modified EPO molecules that can be manufactured and purchased at lower costs.
Another alternative is to develop and test neurochemically distinct compounds with similar downstream effects on neuroplasticity and cognition. A fourth limitation is that EPO treatment requires careful monitoring by a physician and blood tests, which is labour intensive and costly. Nevertheless, it is conceivable that these clinical limitations may be outweighed by the potential cognitive and associated socio-occupational benefits of EPO treatment in patients with a more chronic illness course and functional disability. It is therefore an essential step for future trials to determine whether the EPO-associated improvement of cognitive performance in mood disorder and associated subjective cognitive improvements translate into greater socio-occupational capacity longer-term.

E. Opportunities and challenges with fMRI application in cognition trials
Given the low approval success rates of about 15% in CNS drug development, the pharmaceutical industry has taken immense losses (see chapter II and (Borsook et al. 2013; DiMasi, 2001)). The cost of drug development for CNS conditions from discovery to phase 2 trials is around 100 million dollars (90 million euros), whereas the cost from discovery to approval after phase 3 trials is approximately one billion dollars (0.9 billion euros) (Borsook et al. 2013). In comparison, an fMRI study of a small group of patients in a phase 2 trial costs less than one million dollars (0.9 million euros). The cost-benefit of implementing fMRI investigation before phase 3 trials can therefore be enormous (Borsook et al. 2013). Specifically, testing the ability of an investigational drug to produce target engagement in a validated, reliable fMRI biomarker model of cognitive enhancement may inform go/no-go decisions and thereby lead to massive savings by discontinuing work on a drug that fails to engage the neuronal target.

Although the use of a neurocircuitry biomarker model in treatment discovery strategies with well-devised fMRI experiments may become a major domain of neuroimaging, there are some fundamental limitations of the fMRI technique that must be considered. Specifically, the reproducibility of the BOLD fMRI response is uncertain: test-retest reliability has not been shown consistently across fMRI paradigms and across different measurement times within the same individuals. For example large within-subject variation in BOLD fMRI signal change across repeated measurements has been observed (Zandbelt et al. 2008). This limits the statistical power for detection of a drug effect in fMRI studies with a repeated-measures design and may thereby mask a potential treatment effect; an issue that is still largely unresolved. Indeed, we observed in the prospective fMRI analyses in the EPO trials that only few regions displayed differential effects of EPO versus saline in neural activity change over time. For example, the hypothesized effect of EPO on working memory-related activity in an a priori region of the right dlPFC could not be confirmed in the analysis of differential neural activity change between drug groups from baseline to the post-treatment assessment, possibly due to great within-subject variability in the dlPFC activity with repeated scanning (papers VII and VIII). Significant dlPFC activity differences between groups during n-back WM performance only emerged in the post-interventional cross-sectional fMRI assessment (paper VIII). Based on this finding, we could not make any strong inferences regarding the effect of EPO in this region. Indeed, cross-sectional
fMRI assessments in randomised controlled studies with carefully matched intervention groups (Harmer, 2006; Miskowiak et al. 2007b; Miskowiak et al. 2007c; Miskowiak et al. 2008; Miskowiak et al. 2010a) could potentially have higher statistical power and thus greater sensitivity for treatment effects on BOLD fMRI response. Nevertheless, further studies are required to clarify the optimal design of proof-of-concept studies and how randomised controlled repeated-measures fMRI studies should be powered (i.e., number of participants needed) to get reasonable effect sizes that are useful for decisions in future treatment development. Notably, emerging evidence indicates that the statistical power of repeated-measures randomised controlled fMRI studies can be enhanced by (i) correcting for the confounding effects of physiological noise (the primary source of fluctuations in the BOLD fMRI signal) in the fMRI analyses, (ii) minimizing participants’ stress during the first scanning session by acclimatization in a mock scanner and (iii) using region of interest (ROI) analysis rather than whole-brain voxel-wise analysis which may be overly conservative for longitudinal designs (Zandbelt et al. 2008).

Another major limitation of fMRI is that it provides only an indirect measure of neuronal activity changes. This may be particularly problematic for demonstrating neurocircuitry target engagement for compounds with global physiological effects in the brain which can confound the interpretation of the signal in the cognition-relevant neurocircuitry (Zandbelt et al. 2008). Indeed, we faced this problem in the EPO studies since EPO is likely to affect BOLD fMRI response in the acute treatment phase because of its haematological actions. To tackle this problem, we postponed the post-treatment fMRI assessment until the red blood cell counts were expected to have normalized, six weeks after treatment completion (and verified the red blood cell normalisation with blood tests at this time). We also implemented a visual stimulation control task with no cognitive demands which enabled examination of whether there were any potential global differences in neural activity (i.e., activity unrelated to cognitive performance) between EPO and saline groups. Given the absence of differences between treatment groups in occipital activity to photic stimuli, we could infer that the observed effects of EPO on task-relevant neural activity were unlikely to be confounded by any global changes in cerebral hemodynamic responses (papers VII and VIII). The golden standard approach would be to apply an even more rigorous measure to quantify and adjust for physiological effects on global hemodynamic responses such as arterial spin labelling (ASL).

F. Future directions for the field
With respect to EPO, the evidence from the present studies for beneficial effects of EPO on cognitive function in mood disorders is preliminary. Therefore it is imperative as a next step to investigate if the pro-cognitive of EPO can be replicated in separate cohorts. In such replication studies, objective cognitive dysfunction should be an inclusion criterion and a global cognition outcome should be selected as primary outcome.

More generally, it would also be interesting to target patients at more chronic illness stages in future studies of EPO or other promising cognition treatments such as vortioxetine, modafinil and
mifepristone, given our demonstration of a small but significant increase in patients’ chances of EPO treatment efficacy on cognition with longer illness duration. Notably, the translation of treatment-related cognitive improvement into greater functional capacity in chronically ill patients may be more difficult due to poor environmental opportunities to use the regained cognitive skills given the lack of work, social isolation etc. In this respect, it would be of principal interest to investigate the effects of a multi-modality approach in which treatment with EPO or another promising candidate compound is combined with a psychological intervention targeting cognition or daily functioning such as CR or FR; this could exert synergistic effects with additional benefits over the pharmacological treatment alone and deserves to be further investigated. Importantly, early intervention for cognitive impairment may prevent chronic courses of illness in line with the staging hypothesis of mood disorders (Cosci and Fava, 2013; Vieta et al. 2011). Studies are therefore warranted to investigate whether an early intervention for cognitive impairment in newly diagnosed patients or even in healthy genetically predisposed individuals can improve socio-occupational functioning and prognosis. Finally, our findings also warrant investigation of add-on treatment with EPO or other neuroplasticity-stimulating compounds for TRD patients who undergo cognitive behavioural therapy to examine if this can help patients break dysfunctional behavioural patterns and enhance their ability to learn new strategies to improve their life quality. Importantly, future cognition trials should employ longer follow-up times than the six weeks in our EPO studies (for example six months) and examine whether treatment-associated improvement of cognition translates into increased socio-occupational improvement longer-term (as indicated by anecdotal evidence from our EPO trials). Such findings could have important implications for patients’ functional recovery, quality of life and future societal costs of mood disorders.

Key methodological insights from the EPO trials are that screening for objective cognitive impairment and selection of a global primary cognition outcome are warranted in future trials. Specifically, this approach may ensure enriched samples with greater scope for improvement and greater statistical power to detect treatment efficacy on cognition. Based on the neuroimaging findings of in the EPO studies, future cognition trials with EPO and other candidate treatments may benefit from implementing fMRI at baseline and early on in treatment (e.g. after two weeks of treatment) to examine whether candidate treatments produce similar target engagement in the PFC and functionally related neurocircuitry and whether this is related to subsequent efficacy on cognition. If such studies find prefrontal target engagement to be valid and reliable neuroimaging biomarker for pro-cognitive effects of different treatments across several neuropsychiatric disorders, this could lead to a break-through in treatment development strategies targeting cognitive dysfunction.

G. Conclusion
Cognitive dysfunction in mood disorders has emerged as a key treatment priority but there are no available treatments with solid and lasting efficacy on cognition. This dissertation identified a
number of common methodological challenges in this emerging field and the lack of a fast efficient biomarker to select among new candidate treatments as the principal reasons for the lack of efficacious cognition treatments. The methodological challenges and possible solutions were valuated and exemplified with the author’s randomised placebo-controlled studies of EPO on cognition and underlying neurocircuitry in mood disorders. A translational approach was used, starting with preclinical EPO studies, then moving on to human proof-of-concept fMRI studies and, finally, clinical phase II trials with longer-term EPO treatment and prospective fMRI assessments. This sequential approach seems feasible for future treatment discovery strategies for compounds that show encouraging effects in animal models as it may bring down the high failure rates of clinical phase 3 studies.

The EPO trials revealed the first preliminary evidence for mood-independent improvement of cognition across UD and BD after eight weeks EPO vs. saline treatment (papers IV, V, IX and X), which was accompanied by reversal of subfield hippocampal volume loss (paper VI) and by increase in dorsal prefrontal activity during episodic encoding and working memory (papers VII and VIII). These findings highlight compounds with neuroprotective and plasticity-enhancing properties like EPO as promising candidate treatments for cognitive dysfunction in mood disorders which deserve further investigation. Since the EPO trials were the first to show replicated (albeit preliminary) treatment-related improvement of cognition across BD an UD, the trials presented a unique possibility for post-interventional analyses of (i) the neuronal underpinnings of treatment-associated cognitive improvement and (ii) the clinical predictors of treatment efficacy. Indeed, the findings provided novel evidence for a putative neurocircuitry-based biomarker model for cognitive enhancement. The trials unveiled a clear importance of baseline cognitive deficits for the chances of treatment efficacy on cognition. This finding was followed up by two studies in UD and BD populations that resulted in validation of two new psychological assessment tools to screen for cognitive impairment in future cognition trials.

Three lessons learnt from the EPO trials seem particularly important for the methodology of future cognition trials in mood disorders: First, screening for cognitive impairment with an objective neuropsychological tool such as the SCIP can help target cognition treatments to patients with the greatest scope for cognitive improvement, thereby ensuring enriched populations and increased statistical power for detection of treatment efficacy. Second, it seems feasible to track treatment efficacy with a global cognitive composite score encompassing several cognitive domains with documented deficits in mood disorders. Third, examination of neuronal underpinnings of EPO-related improvement in cognition highlights target engagement in the dorsal PFC and functionally related regions as a putative circuitry-based biomarker model for cognitive enhancement. Future proof-of-concept trials using different promising candidate cognition treatments are warranted to examine whether such ‘prefrontal target engagement’ is a valid biomarker model (Harmer et al. 2010), which would pave the way for a much-needed neuroimaging biomarker tool to screen and select novel cognition treatments. This could lead to a break-through in treatment development strategies by introducing small, relatively inexpensive
fMRI biomarker studies as a crucial *intermediate* step between animal studies and large-scale clinical phase 3 trials. By informing go/no-go decisions in treatment development, fMRI may improve the success rates of phase 3 trials and thereby advance treatment options targeting cognition in mood disorders.
VII. Summary

Cognitive dysfunction, including memory and concentration difficulty, is an emerging treatment target across several neuropsychiatric disorders including unipolar depression (UD) and bipolar disorder (BD). Evidence indicates that deficits in neuroplasticity underlie both cognitive deficits and affective symptoms in these mood disorders. Novel candidate treatments with rapid and enduring effects on neuroplasticity may therefore target both cognition and mood disturbances. However, there are no available treatments with enduring effects on cognition in mood disorders, possibly because of methodological challenges that impede the success of cognition trials.

This dissertation revealed several major methodological challenges in cognition trials in mood disorders, including the lack of consensus on (i) the need to screen for cognitive impairment, (ii) whether to use subjective or objective screening criteria and (iii) which primary cognition outcome measure(s) to select for the assessment of treatment efficacy. A more fundamental methodological problem is the lack of insight into whether candidate cognition treatments target the aberrant activity in cognition-relevant neural circuitries.

The multifunctional growth factor erythropoietin (EPO) was identified as one of the most promising candidate treatments to target deficits in neuroplasticity and cognition in mood disorders. Based on this, two randomised, double-blind, placebo-controlled trials were conducted to investigate the effects of EPO on cognitive deficits and mood symptoms in UD and BD and of the underlying neuronal mechanisms using functional magnetic resonance imaging (fMRI). The EPO trials provide for the first time promising -albeit still preliminary- evidence for a candidate treatment with replicated effects on cognitive impairment across UD and BD. This evidence and methodological implications for the field are outlined in the dissertation. Specifically, the findings of the EPO trials provide novel insight into a putative neurocircuitry biomarker model for cognitive enhancement, which after further validation may facilitate the development of novel cognition treatments. The results also highlight the need to screen for cognitive impairment with objective neuropsychological tests and to select a global cognition composite to track treatment efficacy in future trials. Based on these findings, we conducted two psychological assessments studies which resulted in validation of new much-needed tools to screen for cognitive impairment in mood disorders.

Taken together, the research in this dissertation is likely to give direction to the field with respect to which promising candidate treatments to further investigate, how to implement fMRI in treatment development and how to improve the trial design. These insights may increase the success rates of future cognition trials with consequent benefits for patients and reduced costs for society.
VIII. Danish summary (resumé)

Kognitive vanskeligheder som hukommelses- og koncentrationsbesvær er et nyt behandlingsmål ved flere neuropsykiatriske lidelser inklusiv unipolar depression (UD) og bipolar lidelse (BD). Forskning peger på, at nedsat neuroplasticitet er en central årsag til både kognitive vanskeligheder og affektive symptomer ved disse lidelser. Behandling med direkte og vedvarende effekt på neuroplasticitet kan derfor muligvis forbedre både kognition og affektive symptomer. Der er imidlertid endnu ingen klinisk anvendt behandling med effekt på kognition ved affektive lidelser, hvilket kan skyldes metodevanskeligheder i de kliniske undersøgelser.

Denne afhandling identificerede adskillige metodeudfordringer i kliniske forsøg inden for feltet, såsom mangel på konsensus om (i) behovet for at screene for kognitive vanskeligheder, (ii) hvorvidt subjektive eller objektive screeningskriterier skal anvendes, og (iii) hvilke(t) mål skal vælges som primær effekt parameter. Et mere fundamentalt metodeproblem er uvisheden om, hvorvidt nye kandidatbehandlinger har virkning på den abnorme aktivitet i kognitions-relevante hjerneområder.

Den multifunktionelle vækstfaktor erythropoietin (EPO) blev identificeret som en af de mest lovende kandidatbehandlinger for deficit i neuroplasticitet og kognition ved affektive lidelser. På baggrund af dette udførte vi to randomiserede, dobbeltblindede, placebokontrollerede studier af virkningen af EPO på kognitive vanskeligheder og affektive symptomer ved UD og BD samt af de underliggende neurale mekanismer ved brug af funktionel magnetisk resonans (fMRI). EPO forsøgene demonstrerede for første gang lovdende -om end stadig præliminær- evidens for en mulig behandling med replicerede virkninger på kognitionsvanskeligheder på tværs af UD og BD. Denne evidens og metodemæssige implikationer for fremtidige kliniske forsøg præsenteres i afhandlingen. Konkret peger resultaterne på en mulig hjernebaseret biomarkørmådel for pro-kognitive virkninger, som efter yderligere validering vil kunne effektivisere udviklingen af nye behandlinger. Resultaterne illustrerer også behovet for at screene for kognitive vanskeligheder med objektive neuropsychologiske tests og for at udvælge en global kognitiv composite score til vurdering af behandlingseffekt i fremtidige forsøg. På baggrund af disse fund udførte vi to psykologiske assessment studier, som resulterede i validering af nye brugbare redskaber til at screene for kognitive vanskeligheder ved affektive lidelser.

Samlet set er det sandsynligt at forskningen, som refereres i denne afhandling, vil blive retningsgivende i fremtidige kognitionsforsøg i forhold til, hvilke lovende kandidatbehandlinger bør videreudforskes, hvordan fMRI kan implementeres, og hvordan studiedesign kan optimeres. Denne viden kan øge succesraten for fremtidige kognitionsforsøg, hvilket vil gavne patienter og reducere de samfundsmæssige omkostninger ved affektive lidelser.
IX. References


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