Cognition and neuroplasticity in the remitted state of unipolar depressive disorder

Bo Jacob Hasselbalch

This review has been accepted as a thesis together with four original papers by University of Copenhagen 12th December 2011 and defended on 13th January 2012.

Tutors: Lars Vedel Kessing, Steen Gregers Hasselbalch & Ulla Benedicte Knorr.

Official opponents: René Klysner, Raben Rosenberg & Stein Andersson.

Correspondence: Psychiatric Centre Copenhagen, Department O (Rigshospitalet), Blegdamsvej 9, 2100 Copenhagen, Denmark

E-mail: bjhasselbalch@hotmail.com

Dan Med J 2015;(6):B5080

THE 4 ORIGINAL PAPERS ARE

1. Hasselbalch BJ, Knorr U, Kessing LV. Cognitive impairment in the remitted state of unipolar depressive disorder: a systematic review. J Affect Disord 2010 Dec;134(1-3):20-31.
2. Hasselbalch BJ, Knorr U, Hasselbalch SG, Gade A, Kessing LV. The cumulative load of depressive illness is associated with cognitive function in the remitted state of unipolar depressive disorder. Eur Psychiatry 2012 Sep;28(6):349-55.
3. Hasselbalch BJ, Knorr U, Hasselbalch SG, Gade A, Kessing LV. Cognitive deficits in the remitted state of unipolar depressive disorder. Neuropsychology 2012 Sep;26(5):642-51.
4. Hasselbalch BJ, Knorr U, Bennike B, Hasselbalch SG, Sondergaard MH, Kessing LV. Decreased levels of brain-derived neurotrophic factor in the remitted state of unipolar depressive disorder. Acta Psychiatr Scand 2012 Sep;126(3):157-64.

1. INTRODUCTION

1.1. Epidemiology of depressive disorder.

Depressive disorder is one of the most prevalent psychiatric disorders (1) and contributes to the largest burden of illness worldwide, accounting for approximately 12% of all total years lived with disability (2). The World Health Organization estimates that second to heart disease, depressive disorder will become the most important cause of disability adjusted life years worldwide by the year 2020 (3). Mood disorders are serious conditions in which patients suffer a higher risk of suicide relative to the general population (4). However, despite the introduction of new treatments, depressive disorder seems to be progressive in nature (5;6).

1.2. The concept of depressive disorder and some current insights into the neurobiological foundation of depression.

Depressive symptoms may represent a transient mood state experienced by all normal individuals at some point in their lives as well as a clinical syndrome which includes abnormalities of mood, neuro-vegetative functions, cognition and psychomotor activity. The concept of depression is as old as medicine itself and to this day, we are still in search of the features which may delineate the pathological state of depression from normal states. The term melancholia was first used by Hippocrates in ancient Greek, 400 B.C. Melancholy (from Greek: black bile) was described as a condition associated with despondency, aversion to food, sleeplessness, irritability and restlessness. Fear and depression were perceived as the prolonged means of melancholia and the psychological manifestation of an underlying perturbation of brain function (7). Aristotle (484-322 B.C.) further introduced the idea of a predisposition to melancholy and attributed this to an excess of black bile, and Galen of Pergamon (131-201 A.D.) established that melancholia is a chronic and recurrent condition (7). The explicit conception of manic- depressive illness as a single disease entity dates back to the mid-nineteenth century with the French psychiatrists Faltret and Baillanger independently and simultaneously putting forward the view that depression and mania represent different manifestations of a single illness (7). The two disorders have some familiarities as the rate of depression is elevated in relatives of patients suffering from bipolar disorder (8) and the most popular theory, supported by some, but not all studies, suggest that the two disorders share some underlying disease liability with bipolar disorder being associated with the more severe or deviant form of illness (8). They are, however, not the same conditions.

Current diagnostic criteria for depression represent a clinical and historical consensus upon the most significant symptoms which characterise the depressive disorder. The current clinical concept of depression is mainly categorical, but also takes into account the severity of illness. The DSM and ICD distinguish between categorical subtypes and to some extent also provide a measure of severity as certain symptoms may be taken to reflect severity directly (e.g. psychotic features). Subsidiary indicators of severity employed in daily practise consist of estimates referring to the course of illness (e.g. episode frequency and duration and the degree of psychosocial impairment) as well as quantifications of the number of symptoms as measured by e.g. depression rating scales. Psychiatric disorders can also be viewed as a continuum in which individuals can express specific phenotypical traits, but fluctuate on the extent of the severity of symptoms, just as well as the classification of the subtypes of depression represents a definition by consensus upon a behavioural extreme. In this sense, disorders could also be viewed as the quantitative extremes of environmental and genetic factors that vary throughout the spectrum (distribution). Current diagnostic systems thus

position clinical depression as a single entity that varies dimensionally. The categorical approach is a fundamental necessity for clinical practise as it provides the indication for treatment intervention, and the dimensional approach may further prove itself useful in research and clinical practise e.g. into the course of illness.

The diagnosis of depression is not based on objective tests, but on a set of symptoms. Thus, contemporary categorization of depressive syndromes is based mostly on symptomatic differences and there is currently no coherent evidence as to what extent the subtypes of depression reflect different underlying pathological states or exhibit treatment specificity. Given the heterogeneity of the symptoms observed in depression, it is likely that the underlying pathophysiology of the disorder and the mechanism by which antidepressants reduce the load of depressive symptoms, involve numerous brain regions and transmitter systems. This view is supported by e.g. recent advances in the field of functional neuroimaging, in which changes in activity in distinct brain regions in depression have been detected, e.g. (9). From knowledge of the functions of distinct brain regions in healthy individuals, some clues to which aspects of depressive psychopathology and pathophysiology to which they may contribute are provided. Thus e.g. the role of the hippocampus in depressive disorder has attracted much attention in recent years, since it may mediate some of the cognitive aspects of depression such as memory impairment. A growing body of evidence has also suggested that episodes of depression may be associated with structural brain changes such as atrophy of the hippocampus (10). This have been proposed to occur due to pathological oversecretion of glucocorticoids and concomitant inhibition of neurogenesis, see e.g. (11). However, it is unclear whether these changes precede, e.g. (12), or succeed onset of illness, e.g. (13). Furthermore, the extent to which such changes are found in other areas of the brain is unclear. Various brain areas operating in interacting circuits may mediate the cognitive as well as the emotional and vegetative symptoms of depression, and the neural circuitries underlying the abnormal states of depression are less comprehensively understood. In addition to this, depressive syndromes often occur in the context of numerous medical conditions, such as e.g. vascular disease (14), and the vulnerability to depression is only partly genetic with additional non-genetic factor such as stress, trauma and even stochastic processes during brain maturation contributing to the causes of depression. Furthermore, the expression of multiple genes, which may mediate the vulnerability (or resilience) to depression, may be subject to epigenetic regulation during the course of illness (see e.g. Eric R. Kandel for a more comprehensive perspective on epigenetic regulation, e.g. (15)). Depression is therefore best understood as the multiplicative of the interactions between genetic predisposition and environmental factors.

In summary, it is not widely appreciated whether abnormalities observed in depression constitute developmental abnormalities, which may confer vulnerability to depression, compensatory changes to other pathological processes, or the reminiscences / scars of prior episodes. Further insight into these questions will partly depend upon studies that delineate the onset of such abnormalities within the course of illness and determine whether they antedate depressive episodes in individuals at high familial risk of depression and whether these abnormalities are augmented during the course of illness and exist beyond the depressive state. This study was dedicated to contribute some more insight into the latter question.

1.3. Towards identification of vulnerability markers for depression: application of the endophenotype concept in psychiatry.

Because of the inherent heterogeneity of psychiatric disorders, strategies have been employed in order to reduce the complex components of behaviour into their component parts, as these may provide clues to the genes involved in the expression of the disorders. As briefly touched upon in the previous section, abnormal neuroanatomical as well as neurophysiological, biochemical, endocrinological or neuropsychological findings often accompany psychiatric illness. Although it has been recognized for more than a century that both genetic and environmental factors confer susceptibility and resilience to disease, the use of this framework for exploring the aetiology of psychiatric disorders is more recent (16). Gottesman and Gould (2003) formulated the idea of utilizing an “endophenotype” in order to provide “simpler clues to the genetic underpinnings than the disease syndrome itself” (16) and adapted criteria previously provided for identification of markers in psychiatric genetics (17) to apply to endophenotypes. The following criteria were put forward: 1) The endophenotype is associated with illness in the population. 2) The endophenotype is heritable. 3) The endophenotype is primarily state-independent (manifests in an individual whether or not illness is active). 4) Within families, endophenotype and illness co-segregate. Furthermore, an additional criterion was proposed for diseases which display complex patterns of inheritance (18) 5) The endophenotype found in affected family members is found in non-affected family members at a higher rate than in the general population. Thus, the endophenotype represents an intermediate phenotype between the genotype and the phenotype, but it should also be emphasized that the putative endophenotypes do not necessarily reflect genetic effects as these may be environmental, epigenetic, or multifactorial in origin (16). However, the bottom line is that heritability and stability (state independence) represent key components of any useful endophenotype (19). Thus, the most consistent biological markers of major depression are proposed as biological endophenotypes including various markers such as REM sleep abnormalities, functional and structural brain abnormalities, dysfunctions in serotonergic, catecholaminergic, hypothalamic-pituitary-adrenocortical axis and Corticotrophin Release Hormon -systems, and intracellular signal transduction endophenotypes (20).

1.4. Cognitive function in affective disorders.

Cognitive dysfunction is a key component of several major psychiatric disorders including schizophrenia (21;22) and bipolar disorder (23-27), but the nature and extent of neurocognitive deficits across psychiatric diagnosis is poorly understood. Hence, meta-analyses of studies comparing cognitive performance in patients with schizophrenia and bipolar disorder have suggested that patients with bipolar disorder generally perform better on neuropsychological tests than patients with schizophrenia (28) and some studies seem to suggest that the differences observed between diagnostic groups are quantitative rather than qualitative in nature (29). It is also possible that deficits in global measures of cognitive function such as intelligence quotient (IQ) or general cognitive ability is more pronounced in schizophrenia compared to bipolar disorder (22). However, despite on-going discussions concerning the nature and extent of global cognitive impairment, some research has also suggested that more specific aspects of cognitive functions (e.g. attention, executive function, verbal learning and memory) may serve as cognitive endophenotypes. These might eventually provide useful in genetic studies of the complex psychiatric disorders.

As described above, an abundance of studies have investigated the nature and specificity of cognitive dysfunction in the euthymic phase of bipolar disorder, however, no consensus in this area seems to exist at present. Concerning the nature and specificity of cognitive dysfunction in the remitted state of unipolar depressive disorder, studies which have assessed these questions are few, and current findings seem to point in different directions. Some studies also suggest that cognitive impairment may be a vulnerability factor for bipolar disorder that is present before the onset of illness (27;30) and worsens as the illness progresses (31-33). The number of episodes of depression and mania, as well as the lifetime spent with illness, seems to contribute to the degree of cognitive dysfunction in bipolar disorder (34-36). Episodes of illness may also be more closely linked to the degree of global cognitive impairment than the total duration of illness (34). This may indicate that episodes of illness themselves may have a detrimental effect on cognitive function. Furthermore, manic episodes seem to be more consistently associated with specific impairments, whereas depressive episodes may be associated with a broader range of impairments (33). Some previous studies have included mixed samples of patients with unipolar and bipolar disorder, but direct comparisons of patient groups suggest that the disorders should be considered separately (37). Much less is known about the possible deteriorating effects of unipolar depression, per se, on cognitive function (see systematic review later).

1.5. The role of brain-derived neurotrophic factor in unipolar depressive disorder.

During recent years, research into the pathophysiology and treatment of depression has directed some of its focus on the intracellular signalling pathways involved in the neuroplastic events that regulate the complex psychological and cognitive processes. A growing body of evidence suggest that impairments of neuroplasticity and cellular resilience might underlie the pathophysiology of depression and that antidepressants and mood stabilizers exert a significant effect on signalling pathways that regulate neuroplasticity and cell survival. These findings may in time provide innovative views on the biological keystones of the depressive disorders. Among various putative biological markers for depression, a role of brain derived neurotrophic factor (BDNF) in the pathophysiology of depression has more recently emerged on the scene. The role of BDNF is intriguing since it seems to play an important part in the brain's allostatic response to stress and the mechanisms of actions of antidepressants. It is furthermore likely that it may act as a mediator or moderator of cognitive dysfunction in depression. BDNF belongs to the neurotrophin family, which additionally consists of a nerve growth factor, neurotrophin-3 and neurotrophin-4, e.g. (38). The neurotrophin family has been associated with a variety of psychiatric disorders (39;40) and neurological and neuroimmunological diseases (41). Depression may also be associated with a disruption of mechanisms that govern cell survival and neural plasticity in the brain (42), and preclinical and clinical studies support the view that reduced expression of BDNF could contribute to the vulnerability to depression (43-45). However, very few studies have investigated BDNF levels in patients with unipolar depressive disorder in a remitted state. Thus, it is uncertain whether peripheral BDNF levels are abnormally low in unipolar depressive disorder independent of the depressive state and whether levels decrease in the course of illness

2. AIM AND HYPOTHESES

The endeavour of the present project was to provide further insight into some of the factors which may mediate the vulnerability for and the long term consequences of depression. The aims of this thesis are to investigate 1) whether cognitive function and levels of brain-derived neurotrophic factor (BDNF) are decreased in the remitted state of unipolar depressive disorder, 2) whether cognitive function are impaired within specific areas, 3) the association between cognitive function / BDNF and prior course of illness. BDNF was chosen due to its putative role as an endophenotype for depression (see section 1.4).

The present thesis includes a systematic review of the literature on cognitive function in the remitted state of unipolar depression as well as a description of a clinical study and its results.

We also wished to investigate whether the HPA-axis awakening response, as measured by salivary cortisol concentrations, is reduced in patients; however, the data obtained was insufficient to assess this hypothesis. Furthermore, a subsample of the patients and healthy control individuals received Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MR) scans, but the results of this part of the study will be reported elsewhere.

Hypotheses tested:

1. Cognitive function is impaired in patients in the remitted state of unipolar depressive disorder compared to healthy control individuals (based on results from the current as well as previous studies).
2. Cognitive impairment is associated with the progression of illness. More specifically, the hypothesis tested is that the cumulative number, cumulative duration and cumulative number and durations of subtypes of prior episodes, respectively, is associated with a decrease in global cognitive function, in which increasing number, durations and more severe manifestations of episodes (according to subtype) predict a greater decrease in global cognitive function.
3. Individuals with unipolar depressive disorder may exhibit more profound impairments in some cognitive domains than others, in the remitted state.
4. Brain-derived neurotrophic factor is reduced in patients in the remitted state of unipolar disorder compared to healthy control individuals and decreases in the course of illness.

3. METHODOLOGY

3.1. Method used in the systematic review.

Studies on cognitive function in the remitted state of unipolar depression were identified by a systematic search of on-line databases and by hand search of original papers published between 1980- November 2009. The study selection process is thoroughly described in the paper (section 2.4 and 4.2.).

3.2. Design of the clinical study.

The design of the clinical study is cross-sectional, including cases and controls, and with retrospective assessment of clinical factors related to the course of illness including information from case notes. Furthermore the study aimed to combine epidemiological, clinical and biological approaches.

3.3. Ethics, approvals, registrations and data management.

Information on the study was presented to the potential participant both orally and in written form according to the latest version of the Declaration of Helsinki and the participant was invited

to bring a relative or friend. It was specified that participation was voluntary and furthermore it was made clear that the participant could withdraw the given consent at any time without consequence for future treatment. Participants received a copy of their rights and signed a written informed consent. The participants were offered to be compensated for loss of income and for any travel expenses.
All the personal data of each participant was kept in a Case Record File which fulfilled the medical doctors´ obligation to keep patient records. Participants were not registered in The Danish Psychiatric Central Research Register or in any local hospital registers.
The Local Ethics Committee (H-KF-2007-0028) data protection agency (J.nr. 2007-41-0146) and the Danish National Board of Health (J.nr. 7-505-29-564/1) endorsed the study.

3.4. Conduction of the study.
The study was conducted from November 2007 - November 2010 at the Psychiatric Centre Copenhagen, Rigshospitalet, Copenhagen University Hospital, Denmark (protocol, planning of logistics, provision of additional funding resources, pilot study, recruitment and clinical interviews, acquirement of case-notes from hospital admissions and private practitioners, laboratory analyses of biomarkers and data registration). All the participants were recruited and assessed, and all the biological samples were obtained by the author of the thesis.

3.5. Identification and inclusion of the participants.

3.5.1. The registers.
All inhabitants in Denmark have a unique individual identification number, which is registered in the Civil Person Register (CPR). All psychiatric admissions are registered in the nationwide Danish Psychiatric Central Register (DPR) (46), including data on ICD-10 diagnoses (47). The DPD contains information about date of admission and discharge, main and auxiliary diagnoses as well as personal information such as name and address etc. The diagnoses provided by the DPR are made by clinicians. To improve the diagnostic reliability among clinicians, Danish specialists in psychiatry have completed courses in ICD-10. The ICD-10 has been used in Denmark since January 1, 1994.

3.5.2. Selection criteria.
Patients were identified via the registers as individuals who at the time of the interview were between forty and eighty years of age, and who formerly had received a diagnosis of depressive disorder (minimum moderate according to ICD-10 diagnostic criteria) at their first discharge from a psychiatric hospital in the region of Zealand, in the period between 1994 and 2002. The healthy control individuals were identified via the CPR as persons living in Zealand with access to the same kind of treatment facilities as the patient group. The healthy control individuals were matched by age and gender with the patients.

3.5.3. Exclusion criteria.
The exclusion criteria were: 1) A score of ≥ 8 on the HDRS and / or evidence of a depressive episode occurring less than 8 weeks before the interview (according to the recommended guidelines (48)). 2) Bipolar disorder and schizophrenia spectrum disorder. 3) Diagnosis of dementia before the onset of the first depressive episode or an auxiliary diagnosis of dementia at first admission for depression. 4) Low pre-morbid IQ with a score <12 on the 50-item Danish Adult Reading Test (DART). 5) Significant general medical illness or history of medical illness likely to have an effect on cognitive performance (e.g. untreated thyroid disease, epilepsy, stroke and significant head trauma) or medication with a potential adverse effect on cognition (e.g. corticosteroids. However, sedatives in low dosage levels and anti-depressants, anti-psychotics and mood stabilizers were allowed). 6) History of alcohol or substance abuse. 7) Treatment with electro-convulsive therapy (ECT) within the last 6 months. 8) Psychiatric admission within the last 3 months. 9) Other ethnic origin than Northern or Central European.

3.6. Procedures and assessments.

3.6.1. Screening procedure and non-participant assessment.
All the identified individuals, still alive and still living in Denmark were sent information about the study which included a form to be filled out in case the individual wished to participate and a stamped envelope in which the formula could be returned. In case of no response, the information was mailed one final time. All individuals who wished to participate were contacted per telephone and a screening interview was performed to assess whether the individual met the requirement for entry. Individuals were informed that the interview contained questions on sensitive information and that it was recommended that the individual was in private surroundings. If this was not the case, an appointment was made for a telephone interview at a later date. Of the non-responders, a proportion of the sample was contacted and asked to participate in a telephone interview about the total number of depressive episodes previously experienced and about cognitive complaints. Assessment of the latter was performed using the Global Deterioration Scale (GDS) (49) (described in section 3.6.4). Again, the same information was given prior to an eventual interview.

3.6.2. Clinical assessment and execution of the interview.
The participants were assessed clinically with interviews starting at 9.00 -12.00 PM, including short breaks if necessary. The participants were assessed with the World Health Organization (WHO) Schedules of Clinical Assessment in Neuropsychiatry (SCAN) (50) interview and the 17-item Hamilton Rating Scale for Depression (HDRS) (51), besides a number of questionnaires (the clinical assessment and instruments used are described in detail in section 3.6.4 and 3.7). In case of uncertainty about the eligibility of an individual for inclusion into the study (primary psychiatric diagnosis or other clinically relevant issues) consensus was obtained between two investigators (the author and LVK). Blood samples and measurement of blood pressure etc. was performed at the end of the interview, and the participants received a small gift in appreciation of their contribution. Subsequently, the participants had a one- hour lunch break in which lunch and drinks were provided. Neuropsychological assessment was performed from 13.00-15.00 AM including short breaks.

3.6.3. Primary outcome: Global cognitive function.
A measure of global cognitive function was obtained by the Cambridge Cognitive Examination (CAMCOG) (52). The CAMCOG is the cognitive part of The Cambridge Examination for Mental Disorders of the Elderly (CAMDEX) (53), which evaluates a broad range of cognitive functions. The CAMCOG can either provide a single composite score of overall cognitive performance or it can be broken down into sub-scores. It has been shown that a measure of a common dimension of global cognitive impairment can be obtained by summing errors made in the different cognitive do-

mains, see e.g. (54). Because the various items of the CAMCOG assess cognitive functions at varying grades of difficulty, one of its major advantages is the ability to detect mild forms of cognitive dysfunction. It has been thoroughly validated in Danish population samples (55-57). The CAMCOG takes approximately half an hour to administer, and absence of ceiling effects in test performance has been reported (58).

3.6.4. Secondary outcome: Specific cognitive functions.
Descriptions of most of the neuropsychological tests used in the neuropsychological test-battery are available in "A compendium of neuropsychological tests" (59), and modifications are noted below. In the present study, grouping of tests which were thought to reflect similar processes were chosen. However, we are aware that performance on most tests is influenced by more than one cognitive process and that there is inconsistency regarding the categorization of tests. Cognitive functions were assessed within the following four major cognitive domains:
1. Attention (and processing speed) included four measures from two separate tests; Trail Making A & B (Trail A, Trail B, Trail B-A) and the Symbol Digit Modalities Test (SDMT). Trail A requires the participant to draw lines sequentially connecting 25 encircled numbers distributed on a sheet of paper. Task requirements are similar for Trail B, except for the fact that the person must alternate between numbers and letters. The score on each part represents the amount of time required to complete the task. The difference score between Trails A and B, Trail B-A controls for the general speed of processing and may therefore be a more pure measure of cognitive control. Symbol Digit Modalities Test (SDMT), which requires the subject to write numbers corresponding to each of nine symbols indicated in a coding key in 90 seconds.
2. Memory (verbal and non-verbal learning and memory) included four measures from three separate tests; Rey Auditory Verbal Learning Test (RAVLT, Category Cued Recall (CCR) and Rey-Osterrieth complex figure (ROCF). RAVLT requires the subject to recall of a list of 15 words. We included the total number of words recalled in trials 1-5 (RAVLT-f) and delayed recall after 30 minutes (RAVLT-d). Category Cued Recall (CCR) is easier because it involves learning words organized in semantic categories, and recall is assisted by these categories. Our version was modified from the original (60) to involve 48 objects in 12 categories, displayed as line drawings. We used both the immediate recall score and a delayed recall score. The Rey-Osterrieth complex figure (ROCF) is a test of visuo-spatial constructional ability and visual memory. We included the three minute delayed recall score.
3. Verbal function included four tests. Familiar Faces (61) requires naming of 28 generally well-known faces; Boston Naming Test with 60 objects in line drawings; and two verbal fluency tests, phonological fluency (words starting with s) and semantic fluency (animals), each with number of words generated in 60 seconds.
4. Executive function included four measures from three tests; Stroop Test, Wisconsin Card Sorting Test (WCST) and Letter-Number Sequencing (LNS). A version of the Stroop Test which has previously been used in depression (62) was used. We included the time to complete the incongruent part and the time to complete the incongruent past minus the time to complete the congruent part as a measure of the interference effect. The scores represent the time to complete the task. Letter-Number Sequencing (LNS) is a working memory test also included in the WAIS-III. The subject is read a combination of numbers and letters and is asked to reproduce the numbers first in ascending order and then the letters in alphabetic order. Finally, from the modified Wisconsin Card Sorting Test (WCST) (63) we used a score based on total errors.

3.6.5. Secondary outcome: Brain-derived neurotrophic factor (BDNF).
We chose to obtain samples from whole blood since it has been shown that samples can safely be stored at −30°C for at least 5 years and that the concentrations do not significantly decrease during this time-span (64). Whole blood samples were drawn at 12 PM in ethylene-diamin-tetra-acetat containing tubes, which were immediately frozen and stored at −80°C. The samples were processed with a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) measuring BDNF protein (ChemiKine™ BDNF Sandwich ELISA kit, Chemicon International, CYT306, Millipore, Billerica, MA, USA). Processing was performed on all samples within one week by a laboratory technician, who was blinded to the diagnoses. In preparation for the ELISA, the whole blood samples were thawed and kept on ice while lysed 1:15x10-3 with 3% Triton X-100 (Sigma®) and sonicated (Ultra Turrax IKA T25). Cell debris was separated by centrifugation at 20.800 g for 10 minutes at 4°C and the supernatants collected and stored at -80°C until further examination. Incubation and washing were conducted according to manufacturer's instructions with wells and reagents from the kit. All samples and standards were prepared in duplicate. On the day of processing, the sample supernatant aliquots were thawed, kept on ice and diluted 1:200 with Sample Diluent. For the standard curve a serial dilution of recombinant human BDNF protein standard was performed with Standard Diluent (0-500 pg/ml). Standards and samples were added to wells pre-coated with rabbit anti-human BDNF polyclonal antibody, which were sealed and incubated overnight on a shaker at 4°C. The following day the wells were drained and washed 4 times by pipetting Wash Buffer forcefully into the wells, flicking the plates vigorously and blotting them on absorbent paper. Each well was then incubated for 2.5 hours with biotinylated mouse anti-human BDNF monoclonal antibody, followed by 1 hour incubation with streptavidin horseradish conjugate solution, and lastly by 15 min incubation with TMB/E solution. Each incubation step was performed with plates sealed, at room temperature and on a shaker. All incubation steps were separated by 4 times washing with Wash Buffer as described above. Immediately after terminating the reaction with Stop solution, the optical density of the wells was analysed in a Bio-Rad MicroPlate Reader at 450 nm (ref 595 nm). A standard curve was generated from the serial BDNF standard dilutions, and BDNF protein concentrations in the samples were extrapolated directly from the standard curve. The inter-assay variation was 5.4% and intra-assay variation was 4.2%.

3.6.6. Description of the additional instruments used in assessments.
The SCAN (50) (http://gdp.ggz.edu/scandocs/) is a semi-structured interview and provides a set of instruments and manuals aimed at assessing, measuring and classifying psychopathology and behaviour associated with the major psychiatric disorders in adult life. It can be used for clinical, research and training purposes and was developed within the framework of The World Health Organization. SCAN has a bottom-up approach where no diagnosis-driven frames are applied in grouping the symptoms. Each symptom is assessed in its own right. It has a proven stability and robustness to differentially assess psychotic and neurotic

states. We used the computerized version which provides a diagnosis based on an algorithm.
The Global Deterioration Scale (GDS) (49) was used to compare cognitive function between participants and non-participants. The GDS, in addition to observer based measures on cognition, provides information on daily- life function and emotion. GDS rating of the included participants was performed at the end of the clinical interview and in non-participants who met the requirements for entry, but did not wish to be enrolled.
Proxy estimates of the premorbid cognitive capacity of the participants were obtained by an estimate of education level, as measured on a 17-point scale (years of school and further education) and by the DART. The Danish Adult Reading Test (DART) was used to provide an estimate of premorbid IQ of the participants. It is the Danish version of the National Adult Reading Test (NART) (65;66) and it tests the correct pronunciation of a list of 50 irregular words. Education was evaluated as the number of school years in the interval 7-17 years: primary school (up to 10 years), adding upper secondary level (maximum 12 years), and adding a university degree (maximum 17 years in all).
The 17-item Hamilton Rating Scale for Depression (HDRS)(51). The HDRS is a semi-structured interview covering emotional as well as vegetative symptoms (including mood, sleep, appetite etc.) experienced within the last week. Scores range from 0-52, with a higher score indicating more severe depressive symptoms. The HDRS has been thoroughly validated (67). It was used as a measure of the severity of depressive symptoms and a cut-off score on the scale was used as an exclusion criterion. The use of a clinical rating scale was considered superior to self-reported rating scales as it is emphasized that evaluation of possible signs of psychopathology can only be done clinically.

3.7. Supplementary notes on the methods used.

3.7.1. Matching.
The healthy control individuals were matched with the patients by use of the registers. Thus, following the successive final inclusions of patients into the study, statistics on the current age and gender distribution of the groups were continuously performed, and information about the study was sent to a number of individuals selected from the CPR. More specifically the healthy control individuals were selected from the register according to the gender and age distribution of the included sample at the present time, and the number of individuals selected was further defined by the current participation rate.

3.7.2. Assessment of euthymia and the number, duration and subtypes of episodes.
As this project was dedicated to investigate the possible existence of abnormalities in cognitive and biological markers beyond the depressive state, a particular emphasis was placed on securing that the participants were in a sustained state of clinical remission. Therefore the recommendations put forward by Frank et al. (48) were adopted using the severity criterion of < 8 on the HDRS along with a duration criterion of >8 weeks asymptomatic (which implies a more sustained remission). The SCAN (50) was also used to further exclude the presence of clinical depression within the last eight weeks and to assess whether other significant symptoms of psychopathology were present. Furthermore, from the recognition that depressive episodes are not only characterised by a number of symptoms, but also by the extent to which the condition interferes with psychosocial function, these factors were taken into account when establishing the timing of the initiation and subsequent recovery from depressive episodes. Additionally, initiation or augmentation of antidepressant treatment was used a subsidiary indicator and the diagnosis were further validated by information obtained from case-files and the registers. The Life-Chart Method (68) was used to further anchor the change points in the course of illness.

3.7.3. Assessment of treatment intervention.
Information on dosage levels were extracted from case files from hospital admissions and out-patients hospital contacts as well as from private practitioners and from the information provided from the participants. Equivalent dosage levels of treatment with antidepressants, anti-psychotics and mood stabilizers received throughout life and current treatment with these psychotropic medications, including sedatives, were calculated using the defined daily-dose system (DDD) as recommended by the WHO for international drug utilization studies. This method has previously been used e.g. (34;69). Dosage levels of psychotropic medication and the time periods in which they were used by the patients were entered into an excel work sheet and subsequently cumulated dosage levels were calculated (Table 3).

Table 3:

Table 3 Past and present treatment intervention in participants with unipolar depressive disorder

Treatment intervention	Present treatment N (%)	Lifetime treatment Mean (SD)[2]
Psychotropic medication (any kind)	55 (62.5%)	3623.6 (3229.7)[3]
Side-effects	12 (13.6%)	-
Selective Serotonin Reuptake Inhibitors (SSRI)	18 (20.5%)	1605.1 (2674.7)
Tri- and tetra cyclic antidepressants (TCA)	13 (14.8%)	654.9 (1375.4)
Dual action antidepressants	16 (18.2%)	1054.9 (1970.8)
Other antidepressants	3 (3.4%)	54.5 (266.1)
Lithium	9 (10.2%)	127.5 (454.1)
Anti-epileptic mood stabilizers	6 (6.8%)	78.1 (330.0)
First generation anti-psychotics	0	6.4 (33.5)
Second generation anti-psychotics	2 (2.3%)	41.5 (197.0)
Sedatives	13 (14.8%)	-
Electro-convulsive therapy (ECT)[4]	-	4.2 (7.5)

[1,4] N=88.[2] Cumulative DDD (defined daily dosages), N=82. [3]Cumulative DDD of all antidepressants, mood-stabilizers and antipsychotic agents.

3.7.4. Assessment of health status.
The registers were used to screen for previous hospital admissions for medical conditions and a brief interview about current and previous medical conditions was conducted. Participants with current complaints received a brief medical examination. In addition to this, routine blood measures as well as measurement of blood pressure height, abdominal circumference etc. were obtained.

3.8. Blinding.
The primary investigator was blinded to the CAMCOG score and the neuropsychological test scores of the participants when the information on the psychiatric history and treatment was obtained, and the investigators performing the neuropsychological tests were blinded to the status of the participant (patient / healthy control individual). Rating with the Global Deterioration scale was performed at the end of the clinical interview.

3.9. Sample size estimation.
The power and sample size calculations were guided by a previous study on the difference in cognitive function measured with the CAMCOG between patients with depressive disorder in the remitted state and healthy control individuals (34). At the time of

the study no previous study had assesses the association between the duration or severity of episodes. Based on the study by Kessing (1998), we anticipated finding a mean difference in CAMCOG score of 8 points (SD: 17) between patients with depressive disorder and healthy control individuals. Using a two-sided risk of type 1 error, α, of 0.05; a type 2 error risk, β, of 10%; (power of 90%) and equal group size, the sample size (N) for was calculated to N = 180 (90 patients and 90 healthy controls; www.stat.uiowa.edu/~rlenth/Power/index.html. Due to a limited number of patients with unipolar depressive disorder and healthy control individuals who lived up to the stringent selection criteria and wished to participate, this number of participants was not possible to include in the final analyses.
By taking into account the duration and subtypes of depressive episodes, sensitivity was increased (e.g. a patient with one depressive episode lasting for a total of eight months would have experienced a greater load of depression than a patient with two episodes lasting for a total of four months). We also used extensive exclusion criteria thereby deselecting individuals who might express cognitive impairments on the basis of trauma or other conditions affecting the CNS (an exception to these criteria was a diagnosis of dementia occurring after the onset of depressive illness as dementia may be an end stage in the course of illness of depressive disorder; see e.g. (70;71)). In this way the effects of shared variance which could have led to underestimation of group differences in statistical analyses were minimized.
In respect to the sample size needed to detect differences in BDNF levels between patients and healthy control individuals etc., data were not available when the study was planned, since at that time no previous study had investigated this association.

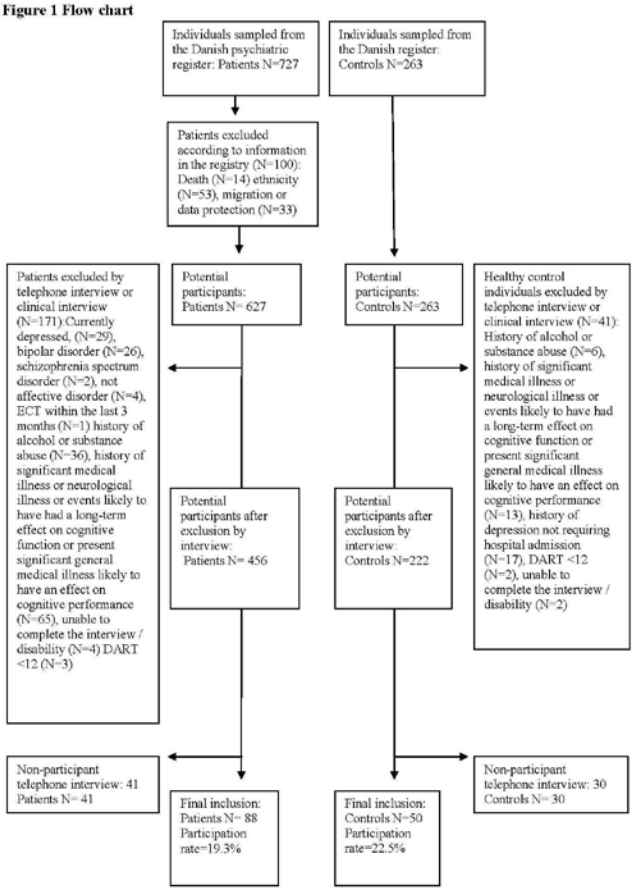
3.10. Statistical methods.
All the statistical analyses were performed by the author under the supervision of the primary supervisor (LVK). All statistical analyses were performed with SPSS version 15.0 and the different statistical techniques used are described in the individual papers.

4. RESULTS.
4.1. Overview.
The results section is divided into two parts: The first part (section 4.2) describes the findings from a systematic review including studies previously conducted within the area. The second part describes the findings of the clinical study, the selection procedure and results of the participant versus non-participant analyses (section 5.3), the characteristics of the included participants (section 5.4) and finally the results of the clinical study are presented in section 5.5, section 5.6 and in section 5.7.

4.2. Cognitive impairment in the remitted state of unipolar depressive disorder: A systematic review (Paper I).
A total of 11 studies were included in the review, including a total of 500 patients who, according to the selection criteria, were defined as remitted from episodes of unipolar depression, and 471 healthy control individuals. In nine of the eleven studies it was found that performance on neuropsychological tests in domains of attention, memory and executive function or in tests providing an estimate on global cognitive function was decreased in patients compared to the healthy control individuals, in at least one of the tests used. Due to the diversity across studies according to the neuropsychological test used, as well as due to the heterogeneity in the clinical spectrum of the patients included, it was not found meaningful to do a meta-analysis in order to be able to further assess, as to what extent the observed impairments could be signs of multiple independent impairments or domain specific cognitive impairments.
In the paper we conclude that methodological drawbacks in studies within this area of research have been prevalent, resulting in limitations in the interpretability of previous findings. It was found that the most prevalent shortcoming seems to be the failure to employ a priori operational criteria, in accordance with recommended guidelines and on the basis of standardised clinical rating scales employing a cut-off criterion as well as a duration criterion in order to evaluate whether patients were truly in a remitted state. This resulted in an inability to validate the clinical status of the patients in a number of studies. Furthermore, some studies failed to take into account important factors which may have an impact on cognitive function, such as age, premorbid cognitive capacity and subclinical depressive symptomatology and to include these factors in the statistical analyses performed. In addition to this, many studies did not provide information on the selection procedure, participation rate and blinding or provided comparative analyses of participants and non-participants, thereby limiting the ability to assess whether selection bias and observer bias may have occurred.
In summary, we conclude that cognitive dysfunction seems to be present in the remitted state of unipolar depressive disorder but that from the findings it was not possible to conclude as to what extent the observed impairments could be signs of multiple independent impairments or domain specific cognitive impairments. Furthermore, very few studies investigated the association between cognition and course of illness, such as e.g. the number of prior episodes and results were divergent. Thus, it was concluded that the association between course of illness and current cognitive function in the remitted state of unipolar depressive disorder is unclear. It was recommended that future studies should focus on disentangling the state and trait characteristics of cognitive dysfunction in unipolar depressive disorder and seek to further clarify the associations with clinical phenotype, course of illness and subsyndromal psychopathology.
Since the conduct of the study, further studies which meet the inclusion criteria employed in the review have been published. Further comments on the findings of these studies can be found in Paper II and III and in the following discussion (section 5)

4.3. Selection and inclusion of the participants and the results of the participant versus non-participant analyses.
From the registers, a total of 627 patients and 263 control individuals were identified, and an invitation to participate in the study was mailed to these individuals. A flow chart is presented in Figure 1 (page 8). Subsequently, 171 patients and 41 control individuals were excluded from the study by clinical interview or telephone interview, resulting in the inclusion of a total of 88 patients and 50 healthy control individuals. Seventy participants, who were eligible for inclusion, but who did not wish to participate in the clinical interview, agreed to participate in a telephone interview (40 patients, 30 healthy control individuals). Thus, we were able to include data from clinical interviews and telephone interviews in 48 % of the patient population and 54 % of control population identified via the registers.

Figure 1 Flow chart



Comparisons were made between participants and non-participants on a number of variables extracted from the registers. As can be seen from Table 1, the participants did not differ in gender, number of previous hospital admissions, age at first admission or on the subtype of the depressive episode at first admission (divided into moderate and severe) from the non-participants. Furthermore, comparisons were made between the participants and the non-participants on the GDS-scores and on the number of previous depressive episodes (according to information obtained from the telephone interviews), and no significant differences were found.

Table 1:

Table 1 Characteristics of the participants versus non-participants.

Register data (patients)	Participants (n=88)	Non-participants (n=368)	p
Gender, females, N (%)	60 (68)	242 (66)	.7
Age at first hospital admission, mean (SD).	49.6 (10.4)	50.8 (11.7)	0.6
Number of previous hospital admissions, mean (SD)	4 (4)	5 (6)	.2
Subtype of the episode at the first hospital admission; moderate, N (%)	42 (48)	184 (50)	.7
Severity of the episode at the first hospital admission; severe, N (%)	46 (52)	184 (50)	.7
Interview data (patients and controls)			
Number of depressive episodes according to the interview; mean (SD)	2.1 (1.3)[1]	1.7 (1.1)[2]	.06
GDS-score (patients), mean (SD)	2.0 (1.3)[1]	1.6 (0.8)[2]	.1
GDS-score (controls), mean (SD)	1.1 (0.3)[3]	1.1 (0.4)[4]	.4

[1]N=84 [2]N=41 [3]N=50 [4]N=30.

4.4. Socio-demographic characteristics of the included participants and clinical characteristics and past and present treatment intervention in the patient group.

The socio-demographic and clinical characteristics of the patient and control group in the total sample are presented in Table 2. The groups were well-matched on age and gender.

Table 2

Table 2.1 Socio-demographic characteristics of patients with unipolar depressive disorder and healthy control individuals.

	Patients (N=88) N (%) /mean (SD)	(Controls N=50) N (%) / mean (SD)
Age	59.8 (9.2)	59.7 (8.0)
Gender (females)	60 (68%)	35 (70%)
HDRS-scores*	2.8 (2.4)*	1.7 (1.7)
DART*	28.6 (8.2)*	32.1 (7.2)
Education (on a 17 point scale)*	12.7 (2.5)*	14 (2.3)
Family history of unipolar disorder in 1st degree relatives*	57 (65%)*	9 (18%)
Family history of bipolar disorder in 1st degree relatives*	5 (6%)	0
Occupational status: employed or student	45 (51%)	34 (68%)
Marital status: married or in a relationship*	54 (61%)*	41 (82%)
Body mass index*	27.2 (4.4)*	25.8 (4.6)
Smoking	24 (24%)	8 (16%)
Regular physical exercise*	46 (54%)*	36 (72%)

*Significant difference between groups ($p<0.05$).

Table 2.2 Clinical characteristics of patients with unipolar depressive disorder

	Mean (SD) / range
Cumulative duration of episodes (months) (N=84)	9.3 (6.2) (2-31)
Number of episodes (N=84)	2.1 (1.3), (1-6)
Number of episodes requiring hospital admission (N=84)	1.5 (0.9), (1-6)
Age at onset (N=88)	47.9 (11.7), (21-72)
Time elapsed since remission from the last depressive episode was achieved (years), (N=88)	6.4 (3.3), (0.4-14)
Duration of illness (years), (N=88)	11.9 (6.5), (6-41)

Mean age was 59.8 (SD 9.2) years in patients and 59.7 (SD 8.0) years in controls and 68% of the patients and 70% of the controls were females. The HDRS-scores among the patients were low (mean 2.8, SD 2.4) although higher than scores among control individuals (mean 1.7, SD 1.7).

The age at onset among the patients was on average 47.9 years (SD 11.7, range 21-72 years) and the duration of illness (years since the first episode of depression) was on average 11.7 years (SD 6.5, range 6-41 years). The time elapsed since the patients had experienced the last depressive episode was on average 6.4 years (SD 3.3, range 0.4-14 years) and only five had experienced an episode within the last year. On average, the patients had experienced 2.1 episodes of depression (SD 1.3, range 1-6 episodes) with an average cumulated duration of 9.4 months (SD 6.2, range 2-31 months), of which on average 1.5 of the episodes required admission to a psychiatric hospital (SD, 0.9, range 1-6 episodes). Statistically significant differences between the groups were present in years of education /17-point scale), premorbid IQ (DART), subclinical depressive symptoms (HDRS), Body Mass Index (BMI), habits of performing regular physical exercise, marital status, and family history of unipolar disorder (only first degree relatives accounted for), (all $p<.05$).

As can be seen from Table 3 (page 6), 62.5% of the patients were currently receiving treatment with psychotropic medication - most commonly Selective Serotonin Reuptake Inhibitors (SSRI). Among the healthy control individuals no one were presently in treatment with, or had previously received treatment with antidepressants, mood-stabilizers or antipsychotic agents. The table furthermore provides information on the cumulative defined daily dosages (DDD) of psychotropic medication that the patients had received through the course of illness (excluding sedatives which it was not possible to secure reliable estimates on), as well as past treatment with Electro Convulsive Therapy (ECT).

4.5. The association between previous episodes of depression and cognitive dysfunction in the remitted state of unipolar depressive disorder (Paper II).

Global cognitive function as measured with CAMCOG was lower in patients (mean score 96.1, SD 4.5) than in control individuals

(mean score 98.4, SD 2.9, z= -3.32, p=0.001). The difference in cognition remained statistically significant in a linear regression model with backward elimination of variables in which age, gender, DART and education were included in the initial model (B=-1.4, 95% C.I. (-2.6,-.16), p=.03). When HDRS scores were introduced in the model, the p-value was only at a borderline level of significance (p=.09). In further analyses, estimates on the clinical variables in 84 out of the 88 patients were included, since in four cases we were not able to obtain reliable information from the clinical interview or from the case-notes on the number or durations of prior episodes. Subsequently, each predictor variable was analysed separately in similar multiple regression models. The cumulative duration of depressive episodes was associated with a -.14 decrease in CAMCOG score per month (=-1.7 per year) depressed (B=-.14, 95% C.I. (-.26,-.02), R2adj= .31, p=.02). In a similar model including the total number of episodes instead of the cumulative duration, the total number of episodes was not significant and was not included in the final model. Further analyses revealed that the decrease in performance on the CAMCOG seemed primarily to be mediated by psychotic episodes in the course of illness; the cumulative duration of psychotic episodes was associated with a -.18 decrease in CAMCOG score per month (= 2.2 per year) depressed (B=-.18, 95% C.I. (-.3,-.04), R2 adj. = .33, p=.01), and the total number of psychotic episodes was associated with a -1.4 decrease in CAMCOG score per psychotic episode (B=-1.4, 95% C.I. (-2.3,-.4), R2 adj. = .35, p=.006). Additionally, we performed repeated analyses wherein the DART was omitted. In these analyses, the previously mentioned associations were confirmed, and in addition to this, the number of episodes requiring hospital admission was associated with a decrease in CAMCOG score; (B=-.9 95% C.I. (-1.7,-.1), R2 adj. = .22, p=.03), (p=.09, with adjustment for DART). Furthermore, no statistically significant associations (Spearman bivariate) were present between the duration of euthymia (years remitted) (p=.1), age at onset (p=.12) or the duration of illness (p=.2) and performance on the CAMCOG. Statistically significant correlations were found between CAMCOG scores and age (rho=-.19, p=.025), education (rho=.41, p<.001), and DART (rho=.52, p<.001) and between CAMCOG scores and HDRS scores in the patient group (rho=-.23, p=.03), but not in the control group (p=1). An association between the cumulative duration of depressive episodes and DART score was present at a borderline level of significance (rho=.20, p=.07).

4.6. Cognitive deficits in the remitted state of unipolar depressive disorder (Paper III).

As can be seen from Table 4, in multiple regression analyses with inclusion of diagnostic group (patient versus healthy control) age, gender, HDRS scores, education and premorbid IQ (DART scores) and with backward elimination of variables, performance was significantly lower in the patient compared to the healthy control group on tests within the domains of attention and visuo-motor speed (Trail-A, Trail-B, Trail B-A and SDMT), and in one of the tests within the domain of executive function (Stroop incongruent) at a Bonferroni corrected significance level of p<.0125. For all other neuropsychological tests, the variable of interest (diagnostic group, patient versus healthy control) was not included in the final model or did not reach the defined level of significance of p=.0125 in the final model, i.e. there was no statistically significant difference between patients and control individuals. Nevertheless, as can be seen from Table 4, there was a trend towards the patient group performing worse than the healthy control group in all the tests.

4.7. Decreased levels of brain derived neurotrophic factor in the remitted state of unipolar depressive disorder (Paper IV).

Mean whole blood BDNF protein concentrations were lower in the patient group (mean 36.8ng/mL, SD 10.8) compared to the healthy control group (mean 43.6 ng/mL, SD 12.7) and a Mann-Whitney U test showed a significant difference between the groups (U=1447, Z=-3.1, p=.002). In a multiple regression model including the covariates age, gender, BMI, education, smoking, physical exercise and HDRS-score, and with backward elimination of variables a diagnosis of unipolar disorder (B=-7.4, 95% C.I. (-11.2, -3.7), p<.001) and age (B= -.2, 95% C.I. (-.4,-.03, p=.02) was negatively associated with BNDF protein levels whereas females exhibited higher levels of BDNF gender (B=7.4, 95% C.I. (3.5, 11.2), p<.001). In further analysis, the interaction term, gender x group was applied to a linear model. The interaction term was not significantly associated with BDNF levels (p=.3), indicating that the diagnosis of unipolar disorder was associated with reduced levels of BDNF, independently of gender. In a similar regression model, no statistically significant associations were found between BDNF levels and the total number of episodes or the cumul ative duration of episodes or the total number or cumulative durations of subtypes of episodes (moderate, severe and severe

Table 4

Table 4 Results of the neuropsychological tests in the patient group compared to the healthy control group

Neuropsychological tests	Patients Median (quartiles), mean (SD)	Controls Median (quartiles) mean (SD)	Multiple regression[1] B[1], 95% C.I, R^2, p[2]
Attention and speed			
Trail-making part A	39 (29,51), 42 (17)	34 (29,39), 36 (12)	7 (2,12), .25, .007
Trail-making part B	88 (63,122), 113 (81)	66 (53,82), 74 (32)	29 (9,49), .33, .005
Trail B-A	48 (30,78), 70 (71)	32 (18,51), 38 (30)	24 (5,41), .32, 0.01
Symbol Digit Modalities Test	40 (30,51), 40 (13)	49 (40,56), 47 (10)	-5 (-8,-2), .50, .003
Verbal learning and memory			
Rey Auditory Verbal Learning Test;			
-immediate recall	45 (37,49), 43 (10)	46 (39,52), 45 (9)	N.s.
-delayed recall	8 (5,12), 8 (5)	9 (8,12), 9 (3)	N.s.
Category Cued Recall;			
-immediate recall	35 (30,38), 34 (6)	36 (32,38), 35 (6)	N.s.
-cued recall	35 (30,38), 33 (6)	33 (30,38), 34 (6)	N.s.
Rey-Osterrieth-Complex-Figure-Test	20 (14,25), 19 (7)	23 (18,26), 21 (6)	N.s.
Verbal function			
Boston naming test	56 (56,58), 55 (5)	58 (56,59), 57 (3)	N.s.
Verbal Fluency; phonetic	15 (12,19), 16 (6)	17 (21,30), 17 (5)	N.s.
Verbal Fluency; semantic	24 (20.29), 24 (7)	26 (21,30), 26 (6)	N.s.
Familiar Faces	18 (16,20), 18 (3)	18 (17,20), 18 (2)	N.s.
Executive function			
Stroop Test;			
-incongruent part	125 (105,125), 134 (45)	110 (95,127), 112 (26)	17 (5,30), .28, .008
-interference score	71 (53,101), 80 (43)	57 (44,77), 62 (25)	N.s.
Wisconsin Card Sorting Test	8 (5,16), 11 (9)	6 (4,12), 9 (7)	N.s.
Letter number sequencing	10 (8,11), 10 (3)	11 (10,12), 11 (2)	N.s.

In backward regression analyses the following covariates were included: Diagnostic group (patient group / control group), age, gender, education, DART and HDRS.

[1] B= unstandardized regression coefficient, C.I. = 95% confidence interval, R^2= adjusted R^2,

[2]p-value. N.s= not significant in the final model at a Bonferroni adjusted p-level of .0125.

with psychotic features according to ICD-10 diagnostic classification), respectively (all p >.05).

5. DISCUSSION

5.1. Main results and comparison with prior studies.

5.1.1. Hypothesis I

As presented in paper I, prior studies seem to suggest that cognitive impairments are present in patients with unipolar disorder in a remitted state. In the present study, impairment of global cognitive function was observed in univariate analysis in patients compared to healthy control individuals (paper II). Nevertheless, a statistically significant difference between residual depressive symptoms and global cognitive function was observed in the patient group and in multivariate analyses, the group difference

was only significant at a borderline level when residual depressive symptoms were additionally controlled for along with the other covariates. Findings from the present and previous studies may therefore suggest that cognitive dysfunction is present in the remitted state in patients with unipolar disorder compared to healthy control individuals, although the specificity of neurocognitive impairment and the association to course of clinical illness is complex in nature- as will be further described in the subsequent sections.

5.1.2. Hypothesis II

As presented in paper II, the results from the study suggest that impairment of global cognitive function is linked to the progression and severity of illness. Thus it was found that the cumulative durations of depressive episodes were associated with the degree of cognitive impairment and that a history of depressive episodes with psychotic features predicted a decrease in global cognitive function to a greater extent than other types of depressive episodes. As presented in the introduction, some studies have suggested that cognitive impairment may be a vulnerability factor for bipolar disorder that is present before the onset of illness (27;30) and worsens as the illness progresses (31-33), and that the number of episodes of depression and mania, as well as the lifetime spent with illness, seems to contribute to the severity of cognitive dysfunction in bipolar disorder (34;35). The mechanism whereby episodes of depression affect cognitive function is widely unknown. However, indirect evidence from brain imaging studies have suggested that repeated and prolonged duration of depressive episodes is associated with permanent structural changes in the CNS such as atrophy of the hippocampus (10) and that significant reductions in hippocampal volume may occur after the onset of illness (13). However evidence within this area is still limited and is hampered by inconsistencies in phenotypic grouping etc. Further it would also seem likely that changes in neurocognitive function could be related to changes in neurotransmitter levels, hormones, intracellular signal transduction pathways or even through altered gene expression.

Even less is known about the possible deteriorating effects of depression per se on cognition in unipolar depressive disorder and, as described in paper I and II, only a few studies have investigated the association between cognitive function and prior course of illness. The association between the duration of depressive episodes and cognitive function have not been investigated until quite recently. Three recent studies (72-74) did not find an association. However, these studies did not specifically aim to investigate the association between the duration of depressive episodes and cognitive function and it is unclear if a systematic screening for past episodes was performed. Furthermore the associations were assessed solely by the use of univariate analyses. Depressive episodes may be also associated with a broader range of impairments in unipolar as compared to bipolar disorder (33) and the depressive illness is a heterogeneous disorder. Therefore, as the studies did not employ an extensive tests-battery or provided a composite score on overall cognitive performance they may have failed to assess aspects of cognitive function that were impaired in the patients as a group by not taking into account that the deficits which may develop in the course of illness may be diffuse in nature. More importantly, the sample sizes in these studies were small (N=20-30), resulting in low statistical power to detect associations between prior course of illness and current cognitive function.

One previous study including patients with bipolar disorder found that the number of months spent with depression or mania was more strongly related to a larger number of neurocognitive domains than number of episodes per se (35). This finding is partly in line with the findings of the present study including patients with unipolar disorder. Episodes of illness may be more closely linked to the severity of global cognitive impairment than the total duration of illness (34). As the cumulative duration of depressive episodes, but not the duration of illness was associated with cognitive function in the present study, this may further strengthen the suggestion that episodes of illness themselves may have a long-term detrimental effect on cognitive function. The question about the underlying mechanisms by which the course of clinical illness is linked to the evolution of cognitive impairment in affective disorder is of additional importance. Kindling is a process by which sensitisation of the brain occurs. The phenomenon was first described by Goddard (1969) in an experimental model for epilepsy in which repeated electroconvulsive stimulation lead to seizures in animals that were initially unresponsive to stimulation (75). Since then kindling has been widely appreciated as an indirect model for the study of neural plasticity, illness progression and pharmacological sensitivity (76) and may serve as a non-homologous model for the evolution of changes over time following repeated episodes of illness in affective disorders. It is also quite feasible that the intensity of depressive episodes contribute to the cumulative load of depressions on cognitive function. Thus, with a sufficient intensity (severity of episodes) to reach the threshold of the brain's reserve capacity, repeated and sustained stimulation (number and duration of episodes) could lead to expression of cognitive impairments linked to underlying permanent changes in brain structure and function.

Cognitive function in patients with psychosis may also represent a deviation from typical cognitive functioning indicating the possible presence of specific abnormalities in development, neuroplasticity and pathophysiology (36), however, no studies prior to this one investigated the association between psychosis in the course of clinical illness in unipolar disorder and cognitive function. It is therefore noteworthy that a history of depressive episodes with psychotic features predicted a decrease in global cognitive function to a greater extent than other types of depressive episodes in the current study. Seemingly, the association between phenotype and cognitive function, and psychosis in particular, requires further attention.

In conclusion, the present findings suggest that prolonged durations of depressive episodes and the presence of psychotic features, in particular, during the course of illness may be associated with deterioration of global cognitive function. Longitudinal studies are evidently the most optimal study design to assess whether cognitive impairment is truly progressive in nature. It is possible that cognitive dysfunction antedates onset of depression and we did not have a direct measure of premorbid cognitive function, however, since we controlled for surrogate measures of premorbid cognitive capacity (education and premorbid IQ; see section 6.3.3, for a further discussion) strengthens the suggestion that depressive episodes in the course of clinical illness contribute significantly to cognitive dysfunction in unipolar depressive disorder.

5.1.3. Hypothesis III

As presented in paper III, patients performed worse than the healthy control individuals on neuropsychological tests which may be indicative of impairment of processing speed and aspects of attention associated with divided attention and mental flexibility (executive control over actions). As previously described, direct

comparison with findings of prior studies are limited by differences in the method used as well as in the patient samples. The majority of previous studies found impairment of attention and / or executive functions in patients compared to control individuals (73;77-82) in at least one of the tests used to access these domains. Findings from longitudinal studies using follow-up testing of depressed patients also suggest that impairments in some aspects of attention and executive functions may not improve at a proportionate level following reduction of depressive symptoms (69;83;84). A previous study by our group found that discrete attentional deficits and executive dysfunctions may be present in the premorbid state in individuals at high risk of developing unipolar disorder (85). In the present study, we employed the same measures of attention; however, we used a different Stroop paradigm. It may be argued, that the findings reflect impairment of aspects of attention and slowing of speed rather than executive functions, since we did not observe a difference on the Stroop interference measure. Oppositely, one may also argue that tests like the verbal fluency tests and, as previously mentioned the Trail B, may involve aspects of executive function. We did not find that the patient group performed worse than the healthy control group on the verbal fluency tests; however, there are some indications of improvement on these tasks following reduction in depressive symptoms (86-88) and it is likely that these tests are particularly sensitive to the clinical state of depression. Furthermore, we did not find that the patients were impaired on measures of verbal function. Presently, there are no indications from studies including patients remitted from depression or from studies including individuals in high genetic risk of developing depression of impairment of verbal functions in unipolar disorder. In a recent study, Behnken et al. (74) found impairment of non-verbal memory function in the delayed condition of the RCFT. We did not find a statistically significant difference between patients and controls on the delayed condition of the RCFT, at the Bonferroni corrected level of significance; however, the p-value was .045, so we cannot completely rule out the possibility of deficits of non-verbal learning and memory.

The majority of previous cross-sectional studies have not suggested that impairment of verbal learning and memory functions is present in patients remitted from unipolar depression (72;74;77;79;80;82). Likewise, longitudinal studies using follow-up testing of depressed patients have also suggested that verbal learning and memory may improve at a significant level in those who achieve remission or respond adequately to treatment, compared to those who do not (83;84). Three previous studies, however, contradict these findings (69;89;90). Several possible explanations for these discrepancies in findings may exist: In the study by Yuan et al. 2009 (89) patients with first episode geriatric depression were included. As late-life depression may be associated with more pronounced memory deficits and poorer response to treatment (91), it is possible that these functions take longer to recover. It is also possible that acquired biological factors are of greater importance in late-onset (LOD) as opposed to early-onset depressive disorder (92;93) and a large number of studies have found a higher rate and severity of white matter hyperintensities (WMH) in patients with LOD compared to healthy elderly individuals (94). However, WMH seems to be associated in particular with executive functions, attention and speed (94), and the association between memory functions and WMH remains unclear. In a study by Neu et al. 2005 (69), no correlation between WMH and performance on the tests used was detected. However, the study was probably not fully powered to assess this association since the number of patients who in fact presented with WMH was small (presumably because the study population was limited to patients with early onset depression). The patients included were of the melancholic subtype and it is also possible that melancholia is associated with specific neuropsychological deficits (95), at least in the depressed state. Accordingly, Austin et al. found that melancholic patients were impaired on mnemonic tasks and tasks of selective attention and set-shifting, while non-melancholic subjects were largely unimpaired (96). Nevertheless, it is not known if the presence of more pronounced deficits in patients with melancholia is a consequence of the melancholic entity itself. Thus, it is possible that residual memory deficits may be present in subgroups of patients with particular clinical features.

In summary, the findings of the present study suggest that patients with unipolar disorder in the remitted state may exhibit cognitive deficits within the domain of attention and that the functional impairment may be characterized by deficits in attention and mental flexibility (executive control over actions). The influence of executive control on attentional processes has been linked to midline prefrontal and frontal areas such as the lateral prefrontal and anterior cingulate cortex (97). The deficits observed are somehow similar to the impairment found in bipolar disorder, see (98) and as attention is a multidimensional concept, the measures of attention employed may not adequately reflect the aspects of attention that are most relevant to unipolar depressive disorder. Thus, it cannot be concluded that the impairment which was observed is specific to unipolar depressive disorder and further studies are needed to characterise these impairments in detail, as well as comparative studies are needed to asses differences in cognitive profiles across psychiatric diagnoses.

5.1.4. Hypothesis IV

As presented in paper IV, whole blood BDNF levels were decreased in patients compared to the healthy control individuals. There was no association between prior course of illness and BDNF levels.

Results from the current study in which BDNF protein concentrations were obtained from whole blood are not directly comparable to prior studies in which BDNF have been measured in plasma or serum. The advantages gained from using whole blood are described in detail in the paper. One prior study including formerly hospitalized out-patients remitted from unipolar depression also found that BDNF levels were lower in patients compared to healthy control individuals (99). A recent study, including a mixed-population sample of patients from mental health care and primary care, did not replicate this finding (100). However, in this study a fraction of the patients were included from primary care. It is therefore possible that no difference was found due to the inclusion of patients who had suffered a less severe manifestation of the disorder than the hospital based sample included in the present study. Furthermore, the study did not utilize clinical assessment possibly including patients suffering from other conditions than unipolar disorder. Neumeister et al. (101) also found equal levels of BDNF in the patient sample compared to the control sample. As the study included unmedicated patients, it is likewise possible that the patients suffered from a more mild manifestation of the disorder than the patients included in the present study. Furthermore, the negative result might be attributed to low sample size. Noteworthy, in this study it was found that during tryptophan depletion, BDNF levels increased in healthy volunteers while remitted patients were unable to mount this presumed compensatory response, thereby suggesting that

dysregulation of BDNF homeostasis in the face of a serotonergic perturbation may contribute to the pathophysiology of depression.

In a study by Kauer-Sant' Anna et al. (102), BDNF levels in patients with bipolar disorder in the early and late stages of the disorder, was compared, showing that BDNF levels were lower in the patients in the late stages of the disorder. It has been proposed that episode-related changes in neurotrophins may explain some of the brain structural changes observed in patients with bipolar disorder (103). We did not find an association with the clinical factors associated with the course of illness (number and durations etc.) and BDNF levels in patients suffering from unipolar disorder. This finding is in concordance with the former mentioned studies by Monteleone et al. and Molendijk et al. However, the finding does not exclude the possibility that BDNF levels might decrease in the course of illness as a consequence of prior depressive episodes in patients with a more severe or recurrent form of illness e.g. as a result of epigenetic alteration of BDNF gene expression.

Since a growing body of evidence has demonstrated that stress decreases the expression of brain-derived neurotrophic factor BDNF in limbic structures that control mood and cognition (104), decreased levels of BDNF could contribute to the atrophy of certain limbic structures, including the hippocampus and prefrontal cortex which have been observed in patients with depressive disorder. Therefore it would seem tempting to assess associations between BDNF levels and cognitive functions. One previous study including patients with bipolar disorder investigated associations between serum BDNF levels and multiple neuropsychological tests and found a statistically significant (positive) correlation between BDNF levels in one of 16 tests in both patients and controls (105). It is possible that this finding may be attributed to type I error due to multiple testing. Whole blood BDNF protein concentrations seem to reflect BDNF levels in the brain quite well (106), however, blood BDNF levels does not reflect the differences in BDNF expression across different brain regions nor does it provide any further substantial clues to the underlying functional impairment. Thus, assessment of associations between BDNF levels and individual neuropsychological tests would not seem to shed any further light on the subject. However, we further investigated if BDNF levels were associated with global cognitive function as measured by the CAMCOG. We did not find an association between BDNF levels and CAMCOG score in either univariate or linear regression analyses, adjusting for age and gender. Furthermore, we found a statistically significant (positive) association between BDNF levels and CAMCOG score in females in the total sample (Spearman's rho =.23, p=. 03), but the difference was not present when analysing the groups separately. Thus, the sample size was not large enough to provide the sufficient statistical power to assess whether levels of BDNF were associated with global cognitive function in patients with unipolar depressive disorder.

Theoretically, in view of other data from areas within the field, the findings may imply that one of the mechanisms underlying impairment of cognitive function may be impairment of cellular resiliency and neuroplasticity due to decreased levels of BDNF. However, the design and the sample size of the study did not permit further evaluation of this possible association and further studies are needed. While addressing the question whether these abnormalities antedate and / or are augmented during the course of illness in unipolar depressive disorder requires further longitudinal studies, our results nevertheless suggest that neurotrophic changes exist beyond the depressive state.

6. STRENGTHS AND LIMITATIONS.

6.1. Sources of information and selection bias.

The motives that the participants expressed for entering the study were diverse: Some expressed an interest in understanding more about the disorder and for others a need to gain further insight into their past and present situation or the experiences their relatives or friends had gone through was the most important motive. Many also said that being part of the study was an obligation that they owed to their fellow human beings. The most frequent reasons for not participating expressed by the patients were that that they did not feel comfortable talking about their past experiences and had decided to leave these memories behind. The most frequent reason for not participating expressed by the controls – which also was the second most frequent reason expressed by the patients- was that they simply did not feel that they had time to spare in order to participate. Since these qualitative reasons for accepting or refusing to participate point in different directions, they do not suggest that the sample selection resulted in inclusion of participants with specific motives or personality traits.

6.1.1. Selection bias.

Selection bias refers to the distortion of a statistical analysis, resulting from the method of collecting the samples. Sample bias occurs when participants are not equally balanced or objectively represented and is a systematic error which may occur due to a non-random selection of a population based sample. This is, however, rarely accounted for in clinical studies. By using the nation-wide Danish Psychiatric Register and the Civil Person Register, the patients and the healthy control individuals were selected according to the same criteria and patients were matched with a healthy control group identified from a population with the same socio-demographic characteristics. Additionally, since the social well-fare system in Denmark is highly developed, the Danish population is socially fairly heterogeneous as a group and treatment is free of charge. In this way, we believe that sampling bias due to socio-demographic factors was minimized. The participant rate in the study was rather low. However, an informal assessment of the degree of selection bias was made by examining associations between background variables and the two primary indicators. As can be seen from Table 1 (page 8), the participants did not differ from the non-participants on a score of cognitive function (GDS) or on age, gender, age at first admission, the severity of the episode at first discharge or in the average number of depressive episodes previously experienced. In conclusion, the effect of a bias in the selection of the participants was minimized.

6.1.2. Observer bias.

Observer bias may influence observer-dependent measures, but not self-rated questionnaires and blood samples. It was intended to blind the investigators performing the neuropsychological tests to the participants' diagnosis. This was not always possible, since the participants sometimes mentioned or provided clues to their diagnosis by mistake. Unfortunately, it was not possible to calculate estimates on inter-rater variability since very few scores were registered systematically. However, the investigators were supervised by an experienced neuropsychologist and inter-ratings were performed on a regular basis.

Concerning blinding to the test scores of the participants, it was not possible to uphold a complete blinding since the interviewer was required to manage the referral to the memory clinic if participants were suspected of having cognitive difficulties based on

the test scores (CAMCOG <95). However, estimates on the number and duration of episodes etc. were secured from the clinical interview before viewing the test scores, and during the later process of validation of information from case notes, blinding was withheld. In summary, the clinical interviews were performed by the same rater, thereby eliminating the possibility of bias due to inter-rater reliability, but introducing the risk of a systematic observer error.

6.1.3. Recall bias.
Recall bias occurs when the way a respondent answers a question, is affected not just by the correct answer, but also by the respondent's memory. The cross-sectional design of the study is particularly vulnerable to this type of bias. However, a systematic approach was used to provide the estimates of the number, duration and the severity (according to ICD-10) of each episode using the SCAN-interview and information was confirmed by case notes from hospital admissions, out-patients contacts and private practitioners. Furthermore, considering the few cases in which it was not deemed possible to obtain reliable estimates, these were not included in the analyses: This applied to patients with the most severe course of illness, and as subsequently revealed after the analyses were performed, were the ones with the lowest performance on the CAMCOG. Excluding these patients may thus have resulted in an underestimation of the strength of the association between prior depressive episodes and current cognitive function.

6.2. Study design.
The cross-sectional design of the study limits the interpretation of the causality of the present findings. Thus, without data on e.g. the premorbid cognitive capacity, premorbid levels of psychopathology and premorbid base-levels of biomarkers, it is difficult to disentangle the contribution of premorbid factors from the contribution of episodes of depression on the outcome measures The association between episodes of depression and BDNF levels and cognitive function, respectively, could be investigated in a longitudinal study with assessment of individuals at risk of depression before onset of illness, during consecutive periods of remission and following successive episodes of depression. However, it should be held in mind that a longitudinal study with inclusion of a large sample of individuals assessed throughout a lifetime would require vast amounts of human and financial resources. The necessity of assessing the impact of environmental exposures from an ever-changing environment and disentangling the effect of these factors from the effect of depression would also prove a substantial challenge. Furthermore, psychiatric diagnoses are contextual in nature and will change over time as new discoveries are made and following changes in the cultural and socio-economic structure of a society.

6.3. The validity of the diagnosis of unipolar depressive disorder.
We aimed to achieve a high diagnostic validity of the diagnosis of unipolar depressive disorder of the patient sample. The Danish Psychiatric Central Research Register (DPR) offers a unique opportunity to identify all individuals with psychiatric admission. It has been shown that the diagnosis of affective disorder in the DPR is correct in 94% of the cases when compared to ICD-10 diagnoses (based on case-notes and interviews) (107). In a recent study, it was also shown that the validity of the diagnosis of depression is highest for severe and moderate type of depression (82.8 for the severe type of single episodes and 76% for the moderate type of single episodes) and decreases for mild episodes (108). Consequently, mild episodes were not taken into account in the present study. Furthermore, in 10-15% of patients with an index diagnosis of depressive disorder, the diagnosis is subsequently changed to bipolar disorder (109). By recruiting the patients via the registers, a unique opportunity to include patients which in many cases had been assessed by clinicians through several hospital admissions was exploited. Furthermore, the diagnosis was validated using the SCAN and by information extracted from clinical case notes. We therefore believe that the validity of the diagnosis of unipolar depressive disorder in the sample is very high compared to other studies conducted within this area.

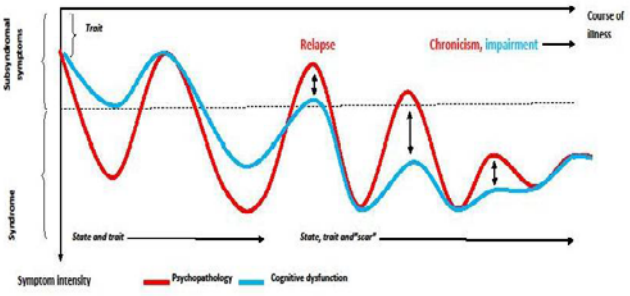
6.4. Confounders.

6.4.1. Age, age at onset and gender.
The design of the study precludes conclusions about the timing of age effects. The ideal solution to disentangle the possible effects of normal aging from illness related changes would be to follow individuals with and without the disorder and identify these changes over time in combination with assessment of concomitant risk factors. However, as can be seen from Table 2 (page 8) there was no significant age difference between the groups. Furthermore, age was included as a covariate in all analyses. As described in paper I and further discussed in the previous section, age of onset may be associated with different risk factors. It is possible that acquired biological factors are of greater importance in late onset of depression as opposed to early onset of depression. It is also possible that early onset is associated with a greater genetic load. However, it does not appear that substantial evidence exists at present, which permits a clear cut distinction between early and late onset depression to be made. In the present study, we included patients with a distribution of age of onset with a wide range (21-72 years). We did not aim to investigate distinctions between early and late onset depression. This would have required a study design in which comparison of well-defined patient groups with early and late onset depression along with e.g. quantification of cerebral vascular pathology and assessment of biological factors associated with normal aging as well as genetic factors. However, we did not find an association between age at onset and e.g. performance on the CAMCOG, suggesting that age of onset did not act as a significant confounder in the present study.
The gender distribution in the sample reflects the known prevalence of depressive disorder according to gender. The literature on gender differences in cognition in unipolar depressive disorder is scarce; however, we included gender in all the statistical analyses performed to eliminate a possible confounding effect. We found that BDNF levels were higher in females in both the patient and the healthy control group and gender differences in BDNF levels may warrant some more attention in further studies.

6.4.2. Somatic illness and substance abuse.
Extensive exclusion criteria were employed in order to exclude patients with current or previous medical conditions which may have confounded the results, and as can be seen from Figure 1 (page 8), a substantial amount of prospects were excluded due to these criteria. Participants underwent a medical interview concerning past and previous medical conditions performed by an MD (the author) with clinical experience in internal medicine, surgery and neurology. Additionally, diagnoses from prior hospital contacts were extracted from the registers and blood samples were obtained. The SCAN was used to exclude substance abuse. Thus, effects of these confounders were minimised.

6.4.3. Premorbid IQ and educational level.
The interpretation of the finding of global impairment of cognitive function is highly dependent on the view on the level of premorbid cognitive function. E.g. some studies including patients with bipolar disorder have suggested that impairment of global intellectual function may be limited to acute episodes (26). If this observation was to hold true, it would predict that factors related to the illness process in itself rather than the trait of bipolar disorder (genetic factors) per se would have an impact on IQ. Premorbid IQ in unipolar depressive disorder has been less comprehensively investigated, and although a body of literature has suggested that premorbid cognitive capacity differs from population norms, when measured using proxy estimates such as educational or occupational level, these estimates are not directly comparable to premorbid IQ measures because of the greater sensitivity of these parameters to environmental factors. In general, high quality prospective studies in relation to unipolar depressive disorder are lacking. However, a prospective study including a population- based sample of 50087 male subjects, using record linkage for hospital admissions during a 27-year follow-up period, found that low IQ may be associated with an increased risk of severe depression (110). Taken together with the fact that it is generally accepted that premorbid IQ estimates such as the National Adult Reading Test (NART) is relatively stable to the effects of illness, it would seem that our finding of a lower premorbid IQ in the patient group compared to the healthy control group may suggest that IQ impairment is a possible trait marker of unipolar depressive disorder. If premorbid IQ then could be considered intrinsic to the disorder, some concerns regarding the use of premorbid IQ as a covariate would be appropriate; this could tend to remove some of the effect of the variables of interest related to the diagnosis if introduced simultaneously in multivariate analyses along with these variables (see e.g. (111)). Furthermore, the DART scores were highly correlated with the CAMCOG scores, which may have lead resulted in an overcorrection of the statistical model. On account of this, we performed repeated analyses in which we excluded the estimate on premorbid IQ as to reduce the risk of type II error. A sample matched on premorbid IQ may have been the ideal solution to overcome this problem, however, this was not intended and such an approach could possibly yield a tendency to select healthy control individuals with a lower cognitive capacity than the average population norm, or the other way around. This could have introduced a systematic error in the form of selection bias.
6.4.4. Subclinical depressive symptoms and duration of remission.
Residual symptoms occur in many depressed patients after acute treatment and may span the typical symptoms of depression, except those characteristic of severe disorders (112). A significant correlation between global cognitive function and levels of residual depressive symptoms was present in the patient group (paper II). However, no significant correlations were present between symptoms and performance on the individual cognitive tests (paper III). As summarized in paper I, prior studies including remitted patients with unipolar depressive disorder as well as studies including patients with bipolar disorder (98) have suggested that mood symptoms, as quantified by observer- based rating scales such as the HDRS and self-reported measures, may be associated with performance on neuropsychological tests even at subclinical levels. Several explanations of these findings are possible. Very few prior studies have employed criteria to assess whether patients were in a sustained state of remission, and it is therefore possible that some prior studies were confounded by the inclusion of patients who were mildly depressed. Secondly, the discrepancies in findings from prior studies in which associations between subclinical mood symptoms and performance on individual cognitive tests were assessed may be attributed to the fact that some cognitive functions quite likely may take longer to recover than others. In our study, we did not find that residual mood symptoms in patients, who on average had been remitted for years, were significantly associated with performance on any of the individual cognitive tests employed. In fact we only found a very modest correlation between residual depressive symptoms and performance on the test of global cognitive function. Notably, a previous study by our group found that twins at high risk of affective disorder seem to present higher levels of subclinical psychopathology than twins with no familial history of affective disorder (113). On balance, it is unclear whether subclinical depressive symptoms may be taken to reflect a static phenomenon intrinsic to the disorder, or whether these symptoms are linked to the progression of illness in which the load of residual psychopathology increases equivalently with illness intensity (frequency and severity of episodes etc.) in the course of illness (An illustration of a hypothetical example, of the possible intrinsic relationship between cognitive function and psychopathology and the interaction between these factors in course of illness is presented in figure 2.

Figure 2 The possible association between cognitive dysfunction, subsyndromal psychopathology and the development of persistent clinically significant residual psychopathology and cognitive impairment in individuals vulnerable to depression



Subclinical psychopathology and cognitive dysfunction may correlate and increase the risk of recurrence and the presence of residual symptoms and thus facilitate the development of persistent clinically relevant core depressive symptoms and cognitive impairment.

The figure also illustrates the inherent methodological problems in disentangling the state from the trait and "scar" characteristics in the chronic state.

These things said there are also some limitations in interpreting the scores on the 17-HDRS as being a direct measure of subclinical depressive psychopathology. Thus, the 6-item subscale scores have a higher validity than the full scale scores (67). However, we chose to include the full scale scores in order to make our findings comparable to the main body of research in which these have been employed. Nevertheless, we believe that controlling for residual depressive symptoms as measured by the HDRS further strengthens our findings of the associations between the clinical factors related to the course of illness and cognitive function.

6.4.5. Medication.
A limitation of the study may be that a proportion of the included patients were medicated during neuropsychological assessment. The impact of psychotropic medications on cognition and the directionality of these effects may differ by the type of medication, cognitive domain of interest and perhaps even diagnosis (36)

and may occur as a direct consequence of the drug's mechanism of action or indirectly through side-effects such as sedation. The latter did not seem to be the case in the current study as none of the participants reported sedation or were treated with psychotropic agents in dosage levels in which sedation could be expected. Further, no statistically significant correlations were found between current treatment with antidepressants, anti-psychotics, sedatives or anti-convulsive mood stabilizers or between lifetime treatment with antidepressants, anti-psychotics, anti-convulsive mood stabilizers, lithium, number of ECTs and global cognitive function as measured by the CAMCOG. We did observe a correlation between present treatment with lithium and impaired performance on the CAMCOG, but this finding could be purely coincidental since only nine patients received treatment with lithium. We did not asses associations between antidepressant treatment and performance on individual cognitive tests since multiple tests were employed and such a strategy could have resulted in positive findings by chance only. Correction for medication in multivariate analyses would introduce a confounder that would tend to remove some of the explanatory power of the primary predictor variables; e.g. according to Danish guidelines for treatment of unipolar disorder, treatment with lithium is recommended for patients with treatment -resistant or more recurrent depression (114). Indeed, we confirmed that there was a correlation between lithium treatment and the cumulative duration of prior episodes. This also underlines the fact that iatrogenic and illness-related factors are not easily disentangled, and our study design did not make it possible to delineate the possible cognitive side-effects of psychotropic medication from factors associated with chronicity. Finally, it may be that polypharmacy and the use of antidepressants as well as sedatives in high dosages may contribute to cognitive impairments in particular. The directionality of the relationship of cause and effect between psychotropic medication and cognitive outcome is uncertain, but it is likely that patients with a more severe course of illness are prescribed a larger number of drugs. To date it has not been possible to study a population of patients with the most severe course of illness who did not receive medication, however, one previous study compared euthymic medicated with unmedicated patients with bipolar disorder on neuropsychological performance and found no difference between the groups when correcting for mood-symptoms, suggesting that deficits are an integral part of the disorder (115). Similar studies including patients with unipolar disorder have not been conducted. In spite of the considerations on the possible limitations of findings from medicated patients the fact is that the body of evidence in this area is scarce (for a comprehensive review see e.g. (116). In fact, very few psychotropic drugs used for treating depression have been associated with cognitive impairment and some findings may even suggest that some antidepressants may enhance neuroplasticity and may have a neuroprotective effect during depression (117;118). It is also possible that antidepressants and lithium may reduce the risk of dementia (119;120) . On balance, and also in view of previous research, we find it unlikely the findings from the present studies are explained by an effect of medication.

6.4.6. Nosocomial factors.

As briefly touched upon in paper I, it is unclear whether cognitive impairment is a factor leading to hospitalisation, or whether factors related to hospitalisation exacerbate cognitive deficits. In the present study (paper II), the total number of admissions was found to be associated with global cognitive function (not significant co-varying for premorbid IQ). It may be argued that hospitalisation may occur for other reasons than mood episodes (121), this finding, however, would suggest that hospital admissions may be an indicator of a more severe manifestation of affective episodes.

6.4.7. Co-morbidity.

Co-morbidity in depression may be due to shared symptoms, true co-morbidity and treatment related co-morbidity. Thus e.g. co-morbidity between anxiety disorders and depression may be explained by shared genetic vulnerability to both disorders (122). We did not introduce e.g. co-morbid diagnosis of anxiety as a covariate in multivariate analyses, as this could have resulted in introduction of type II error as a consequence of shared variance. Other study designs are needed to investigate such associations, in which clear cut depressive phenotypes with and without co-morbid disorders are compared.

6.4.8. Family history of affective disorder.

The inclusion of healthy control individuals with a family history of affective disorder may have resulted in underestimation of the differences between the groups. However, considering the high prevalence and the, presumably, substantial genetic heterogeneity of depressive disorder, excluding healthy control individuals with a family history of depression could also have resulted in the inclusion of a super healthy control group. Since the diagnostic validity of the diagnosis of depression in first degree relatives (in which information was obtained second hand from the patients) was considered low, sub-analyses including these parameters were not performed.

6.5. Representativeness.

Our participants were Caucasians of Northern European descent. The control individuals were matched according to age and gender with the patients and were randomly selected from a representative population sample. The included patients had previously been hospitalized for depression in the Region of Zealand in Denmark and they did not differ from the patients who did not participate on a rating scale of cognitive function (GDS) or on age, gender, age at first admission, severity of the episode at first discharge or in the average number of depressive episodes experienced (paper 2). In this way, we believe that our findings can be generalised to patients treated for unipolar depressive disorder according to ICD-10 who have been hospitalised at least once with a diagnosis of depression of at least moderate degree. The study cannot be generalised to younger patients and milder forms of depressive disorder.

7. CONCLUSIONS.

Global cognitive dysfunction may develop in the clinical course of unipolar depressive disorder. Episodes of illness themselves seem to contribute to the degree of cognitive dysfunction and a history of depressive episodes with psychotic features may confer an increased risk of cognitive impairment. Furthermore, cognitive deficits are present in the remitted state of unipolar depressive disorder and seem to reside more within the cognitive domain of attention than within other domains, and may be further characterised by impairment of processing speed and divided attention, although this needs confirmation in future studies and in other patient samples. Patients with unipolar depressive disorder also seem to have abnormally low peripheral levels of brain-derived neurotrophic factor that persists beyond the depressive state. Low levels of BDNF may be a vulnerability factor for depression

and may contribute to cognitive dysfunction due to impairment of cellular resiliency and neuroplasticity. However, further studies are required.

8. IMPLICATIONS.

The preferred approach to study the development of cognitive impairment would be a prospective study in which individuals at high risk of developing unipolar depressive disorder are followed longitudinally. To date no such study exists. The present findings suggest that cognitive impairment in unipolar depressive disorder is neurodevelopmental as well as neurodegenerative in nature. Clearly, further longitudinal studies are required in order to disentangle the individual contribution of these components to cognitive dysfunction. Furthermore, as our findings suggest that episodes of depression may have a deteriorating effect of cognitive function, there is an urgent need to further investigate whether pharmacological treatment, cognitive remediation and early and sustained intervention decreases the risk of cognitive decline. Finally, while unipolar depressive disorder is significantly associated with genetic factors, genes alone are not sufficient for individuals to express the illness. Thus, the impact of environmental and clinical risk factors on cognitive function requires further attention. Whatever the details are, it is also quite possible that cognitive dysfunction and impairment of neuroplasticity may represent endophenotypic abnormalities. The significance of the interaction between these factors needs further attention.

It is well known that cognitive impairment has consequences for psychosocial function and quality of life. Attention is close to what laymen would consider to be the ability to concentrate, and it is usually assumed to form the basis for more complex cognitive tasks requiring effortful processing of external stimuli. It therefore seems reasonable to presume that the cognitive deficits expressed by individuals suffering from unipolar disorder, may be a limiting factor in their everyday life. The results from the study suggest that these problems may persist after a substantial period in remission and increases in the course of clinical illness. Little is known about the possible effect of cognitive rehabilitation. This area may deserve more attention and it would be tempting to establish cognitive rehabilitation programs, in order to facilitate patients returning to their respective sphere of work and social life, as close as possible to their premorbid level of functioning.

9. SUMMARY

It is widely unknown whether the remitted state of unipolar depressive disorder is characterized by abnormalities in cognition and neuroplasticity and whether such changes are associated to the prior course of illness. The aims of the present thesis is to investigate 1) whether global cognitive function and levels of brain-derived neurotrophic factor (BDNF) in whole blood are decreased in the remitted state of unipolar disorder, 2) whether cognitive function is impaired within specific areas, 3) the association between cognitive function / BDNF and prior course of illness.

The study is a cross sectional case control study, identifying participants via Danish registers. Patients were identified via the Danish Psychiatric Central Register as individuals who presently were between forty and eighty years of age, and who formerly had received a diagnosis of depressive disorder (with minimum moderate severity) at their first discharge from a psychiatric hospital in the region of Zealand, in the period between 1994 and 2002. Healthy control individuals, matched by age and gender with the patients, were identified via the Civil Person Register (CPR) as persons living in Zealand with access to the same kind of treatment facilities as the patient group. Clinical assessment of diagnosis, prior course of illness and current psychiatric status was done in an extensive interview. Neuropsychological testing, including a measure of global cognitive function, the Cambridge Cognitive Examination (CAMCOG), was done independently and blinded for psychiatric status (patient versus healthy control). Only individuals with a score of ≤7 on the 17-item Hamilton Depression Rating Scale and who did not experience a depressive episode less than 8 weeks before the interview were included in the study.

Compared to the healthy control individuals, global cognitive function, as measured with the CAMCOG, was lower in patients. The cumulative duration of prior depressive episodes as well as a history of psychotic depression was associated with cognitive impairment. Furthermore, cognitive deficits seemed to reside more within the cognitive domain of attention than within other domains, and was characterised by impairment of processing speed and divided attention. Patients with unipolar depressive disorder had reduced blood levels of BDNF but no association was found to prior course of illness. Finally, global cognitive function was not associated with levels of BDNF.

Further studies are needed to characterize the association between cognitive dysfunction in unipolar disorder and neuroplasticity

REFERENCES

1. Olsen LR, Mortensen EL, Bech P. Prevalence of major depression and stress indicators in the Danish general population. Acta Psychiatr Scand 2004 Feb;109(2):96-103.
2. Ustun TB, Ayuso-Mateos JL, Chatterji S, Mathers C, Murray CJ. Global burden of depressive disorders in the year 2000. Br J Psychiatry 2004 May;184:386-92.
3. Murray CJ, Lopez AD. Evidence-based health policy--lessons from the Global Burden of Disease Study. Science 1996 Nov 1;274(5288):740-3.
4. Bostwick JM, Pankratz VS. Affective disorders and suicide risk: a reexamination. Am J Psychiatry 2000 Dec;157(12):1925-32.
5. Kessing LV, Andersen PK, Mortensen PB, Bolwig TG. Recurrence in affective disorder. I. Case register study. Br J Psychiatry 1998 Jan;172:23-8.
6. Kessing LV, Hansen MG, Andersen PK. Course of illness in depressive and bipolar disorders. Naturalistic study, 1994-1999. Br J Psychiatry 2004 Nov;185:372-7.
7. Goodwin FK, Jamison KR. Manic-Depressive Illness. Oxford University Press; 2007.
8. Fava M, Kendler KS. Major depressive disorder. Neuron 2000 Nov;28(2):335-41.
9. Malhi GS, Lagopoulos J. Making sense of neuroimaging in psychiatry. Acta Psychiatr Scand 2008 Feb;117(2):100-17.
10. Sheline YI, Sanghavi M, Mintun MA, Gado MH. Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. J Neurosci 1999 Jun 15;19(12):5034-43.
11. Sapolsky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 2000 Oct;57(10):925-35.
12. Baare WF, Vinberg M, Knudsen GM, Paulson OB, Langkilde AR, Jernigan TL, et al. Hippocampal volume changes in

healthy subjects at risk of unipolar depression. J Psychiatr Res 2010 Jan 20.
13. McKinnon MC, Yucel K, Nazarov A, MacQueen GM. A meta-analysis examining clinical predictors of hippocampal volume in patients with major depressive disorder. J Psychiatry Neurosci 2009 Jan;34(1):41-54.
14. Alexopoulos GS. The vascular depression hypothesis: 10 years later. Biol Psychiatry 2006 Dec 15;60(12):1304-5.
15. Kandel ER. A new intellectual framework for psychiatry. Am J Psychiatry 1998 Apr;155(4):457-69.
16. Gottesman II, Gould TD. The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 2003 Apr;160(4):636-45.
17. Gershon ES, Goldin LR. Clinical methods in psychiatric genetics. I. Robustness of genetic marker investigative strategies. Acta Psychiatr Scand 1986 Aug;74(2):113-8.
18. Leboyer M, Bellivier F, Nosten-Bertrand M, Jouvent R, Pauls D, Mallet J. Psychiatric genetics: search for phenotypes. Trends Neurosci 1998 Mar;21(3):102-5.
19. Gould TD, Gottesman II. Psychiatric endophenotypes and the development of valid animal models. Genes Brain Behav 2006 Mar;5(2):113-9.
20. Hasler G, Drevets WC, Manji HK, Charney DS. Discovering endophenotypes for major depression. Neuropsychopharmacology 2004 Oct;29(10):1765-81.
21. Heinrichs RW, Zakzanis KK. Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. Neuropsychology 1998 Jul;12(3):426-45.
22. Keefe RS, Fenton WS. How should DSM-V criteria for schizophrenia include cognitive impairment? Schizophr Bull 2007 Jul;33(4):912-20.
23. Quraishi S, Frangou S. Neuropsychology of bipolar disorder: A review. Journal of Affective Disorders 2002;72(3): 209-226:-226.
24. Robinson LJ, Thompson JM, Gallagher P, Goswami U, Young AH, Ferrier IN, et al. A meta-analysis of cognitive deficits in euthymic patients with bipolar disorder. J Affect Disord 2006 Jul;93(1-3):105-15.
25. Torres IJ, Boudreau VG, Yatham LN. Neuropsychological functioning in euthymic bipolar disorder: a meta-analysis. Acta Psychiatr Scand Suppl 2007;(434):17-26.
26. Balanza-Martinez V, Rubio C, Selva-Vera G, Martinez-Aran A, Sanchez-Moreno J, Salazar-Fraile J, et al. Neurocognitive endophenotypes (endophenocognitypes) from studies of relatives of bipolar disorder subjects: a systematic review. Neurosci Biobehav Rev 2008 Oct;32(8):1426-38.
27. Bora E, Yucel M, Pantelis C. Cognitive endophenotypes of bipolar disorder: a meta-analysis of neuropsychological deficits in euthymic patients and their first-degree relatives. J Affect Disord 2009 Feb;113(1-2):1-20.
28. Krabbendam L, Arts B, van OJ, Aleman A. Cognitive functioning in patients with schizophrenia and bipolar disorder: a quantitative review. Schizophr Res 2005 Dec 15;80(2-3):137-49.
29. Stefanopoulou E, Manoharan A, Landau S, Geddes JR, Goodwin G, Frangou S. Cognitive functioning in patients with affective disorders and schizophrenia: a meta-analysis. Int Rev Psychiatry 2009;21(4):336-56.
30. Arts B, Jabben N, Krabbendam L, Van OJ. Meta-analyses of cognitive functioning in euthymic bipolar patients and their first-degree relatives. Psychol Med 2008 Jun;38(6):771-85.
31. Altshuler LL. Bipolar disorder: are repeated episodes associated with neuroanatomic and cognitive changes? Biol Psychiatry 1993 Apr 15;33(8-9):563-5.
32. Martinez-Aran A, Vieta E, Reinares M, Colom F, Torrent C, Sanchez-Moreno J, et al. Cognitive function across manic or hypomanic, depressed, and euthymic states in bipolar disorder. Am J Psychiatry 2004 Feb;161(2):262-70.
33. Robinson LJ, Ferrier IN. Evolution of cognitive impairment in bipolar disorder: a systematic review of cross-sectional evidence. Bipolar Disord 2006 Apr;8(2):103-16.
34. Kessing LV. Cognitive impairment in the euthymic phase of affective disorder. Psychol Med 1998 Sep;28(5):1027-38.
35. van Gorp WG, Altshuler L, Theberge DC, Wilkins J, Dixon W. Cognitive impairment in euthymic bipolar patients with and without prior alcohol dependence. A preliminary study. Arch Gen Psychiatry 1998 Jan;55(1):41-6.
36. Lewandowski KE, Cohen BM, Ongur D. Evolution of neuropsychological dysfunction during the course of schizophrenia and bipolar disorder. Psychol Med 2011 Feb;41(2):225-41.
37. Paradiso S, Lamberty GJ, Garvey MJ, Robinson RG. Cognitive impairment in the euthymic phase of chronic unipolar depression. Journal of Nervous and Mental Disease 1997;185(12): 748-754:-754.
38. Dawbarn D, Allen SJ. Neurotrophins and neurodegeneration. Neuropathol Appl Neurobiol 2003 Jun;29(3):211-30.
39. Post RM. Role of BDNF in bipolar and unipolar disorder: clinical and theoretical implications. J Psychiatr Res 2007 Dec;41(12):979-90.
40. Brunoni AR, Lopes M, Fregni F. A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. Int J Neuropsychopharmacol 2008 Dec;11(8):1169-80.
41. Schulte-Herbruggen O, Braun A, Rochlitzer S, Jockers-Scherubl MC, Hellweg R. Neurotrophic factors--a tool for therapeutic strategies in neurological, neuropsychiatric and neuroimmunological diseases? Curr Med Chem 2007;14(22):2318-29.
42. D'Sa C, Duman RS. Antidepressants and neuroplasticity. Bipolar Disord 2002 Jun;4(3):183-94.
43. Duman RS, Heninger GR, Nestler EJ. A molecular and cellular theory of depression. Arch Gen Psychiatry 1997 Jul;54(7):597-606.
44. Duman RS. Neuronal damage and protection in the pathophysiology and treatment of psychiatric illness: stress and depression. Dialogues Clin Neurosci 2009;11(3):239-55.
45. Hashimoto K. Brain-derived neurotrophic factor as a biomarker for mood disorders: an historical overview and future directions. Psychiatry Clin Neurosci 2010 Aug;64(4):341-57.
46. Munk-Jorgensen P, Kastrup M, Mortensen PB. The Danish psychiatric register as a tool in epidemiology. Acta Psychiatr Scand Suppl 1993;370:27-32.
47. The Danish National Board of Health. Classification of diseases. The Danish edition of ICD-10. http://www sst dk 1992.
48. Frank E, Prien RF, Jarrett RB, Keller MB, Kupfer DJ, Lavori PW, et al. Conceptualization and rationale for consensus definitions of terms in major depressive disorder. Remission, recovery, relapse, and recurrence. Arch Gen Psychiatry 1991 Sep;48(9):851-5.

49. Reisberg B, Ferris SH, de Leon MJ, Crook T. Global Deterioration Scale (GDS). Psychopharmacol Bull 1988;24(4):661-3.
50. Wing JK, Babor T, Brugha T, Burke J, Cooper JE, Giel R, et al. SCAN. Schedules for Clinical Assessment in Neuropsychiatry. Arch Gen Psychiatry 1990 Jun;47(6):589-93.
51. HAMILTON M. A rating scale for depression. J Neurol Neurosurg Psychiatry 1960 Feb;23:56-62.
52. Roth M, Huppert RM. CAMDEX-R: The Cambridge Cognitive Examination for the elderly -revised. Psykologisk Forlag, Copenhagen 2002.
53. Roth M, Tym E, Mountjoy CQ, Huppert FA, Hendrie H, Verma S, et al. CAMDEX. A standardised instrument for the diagnosis of mental disorder in the elderly with special reference to the early detection of dementia. Br J Psychiatry 1986 Dec;149:698-709.
54. Wouters H, van Gool WA, Schmand B, Zwinderman AH, Lindeboom R. Three sides of the same coin: measuring global cognitive impairment with the MMSE, ADAS-cog and CAMCOG. Int J Geriatr Psychiatry 2010 Aug;25(8):770-9.
55. Andersen K, Lolk A, Nielsen H, Andersen J, Olsen C, Kragh-Sorensen P. Prevalence of very mild to severe dementia in Denmark. Acta Neurol Scand 1997 Aug;96(2):82-7.
56. Andersen K, Nielsen H, Lolk A, Andersen J, Becker I, Kragh-Sorensen P. Incidence of very mild to severe dementia and Alzheimer's disease in Denmark: the Odense Study. Neurology 1999 Jan 1;52(1):85-90.
57. Lolk A, Nielsen H, Andersen K, Andersen J, Kragh-Sorensen P. CAMCOG as a screening instrument for dementia: the Odense study. Cambridge Cognitive Examination. Acta Psychiatr Scand 2000 Nov;102(5):331-5.
58. Verhey FR, Huppert FA, Korten EC, Houx P, de VM, van LN, et al. Cross-national comparisons of the Cambridge Cognitive Examination-revised: the CAMCOG-R: results from the European Harmonization Project for Instruments in Dementia. Age Ageing 2003 Sep;32(5):534-40.
59. Strauss E, Sherman E, Spreen O. A compendium of neuropsychological tests: Administration, norms and commentary (3 ed.) . New York: Oxford University Press 2006.
60. Buschke H, Sliwinski MJ, Kuslansky G, Lipton RB. Diagnosis of early dementia by the Double Memory Test: encoding specificity improves diagnostic sensitivity and specificity. Neurology 1997 Apr;48(4):989-97.
61. Waldemar GBPSEKMLNA&POB. Cognitive profiles and regional cerebral blood flow patterns in dementia of the Alzheimer type. European Journal of Neurology 1994 Sep 1;(1):81-9.
62. Ravnkilde B, Videbech P, Clemmensen K, Egander A, Rasmussen NA, Rosenberg R. Cognitive deficits in major depression. Scand J Psychol 2002 Jul;43(3):239-51.
63. Nelson HE. A modified card sorting test sensitive to frontal lobe defects. Cortex 1976;12:313-24.
64. Trajkovska V, Marcussen AB, Vinberg M, Hartvig P, Aznar S, Knudsen GM. Measurements of brain-derived neurotrophic factor: methodological aspects and demographical data. Brain Res Bull 2007 Jun 15;73(1-3):143-9.
65. Nelson HE, O'Connell A. Dementia: the estimation of premorbid intelligence levels using the New Adult Reading Test. Cortex 1978;14:234-44.
66. Crawford JR, Besson JA, Parker DM, Sutherland KM, Keen PL. Estimation of premorbid intellectual status in depression. Br J Clin Psychol 1987 Nov;26 (Pt 4):313-4.
67. Elsass P, Ivanouw J, Mortensen EL, Poulsen S, Posenbaum B. Assessmentmetoder. Håndbog for psykologer of psykiatere. 1, 475-492. 2006. Psykologisk Forlag A/S. Ref Type: Edited Book
68. Post RM, Roy-Byrne PP, Uhde TW. Graphic representation of the life course of illness in patients with affective disorder. Am J Psychiatry 1988 Jul;145(7):844-8.
69. Neu P, Bajbouj M, Schilling A, Godemann F, Berman RM, Schlattmann P. Cognitive function over the treatment course of depression in middle-aged patients: correlation with brain MRI signal hyperintensities. J Psychiatr Res 2005 Mar;39(2):129-35.
70. Kessing LV, Andersen PK. Does the risk of developing dementia increase with the number of episodes in patients with depressive disorder and in patients with bipolar disorder? J Neurol Neurosurg Psychiatry 2004 Dec;75(12):1662-6.
71. Ownby RL, Crocco E, Acevedo A, John V, Loewenstein D. Depression and risk for Alzheimer disease: systematic review, meta-analysis, and metaregression analysis. Arch Gen Psychiatry 2006 May;63(5):530-8.
72. Pedersen A, Kuppers K, Behnken A, Kroker K, Schoning S, Baune BT, et al. Implicit and explicit procedural learning in patients recently remitted from severe major depression. Psychiatry Res 2009 Aug 30;169(1):1-6.
73. Bhardwaj A, Wilkinson P, Srivastava C, Sharma M. Cognitive deficits in euthymic patients with recurrent depression. J Nerv Ment Dis 2010 Jul;198(7):513-5.
74. Behnken A, Schoning S, Gerss J, Konrad C, de Jong-Meyer R, Zwanzger P, et al. Persistent non-verbal memory impairment in remitted major depression - caused by encoding deficits? J Affect Disord 2010 Apr;122(1-2):144-8.
75. Goddard GV, McIntyre DC, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. Exp Neurol 1969 Nov;25(3):295-330.
76. Weiss SR, Post RM. Kindling: separate vs. shared mechanisms in affective disorders and epilepsy. Neuropsychobiology 1998 Oct;38(3):167-80.
77. Weiland-Fiedler P, Erickson K, Waldeck T, Luckenbaugh DA, Pike D, Bonne O, et al. Evidence for continuing neuropsychological impairments in depression. J Affect Disord 2004 Oct 15;82(2):253-8.
78. Paelecke-Habermann Y, Pohl J, Leplow B. Attention and executive functions in remitted major depression patients. J Affect Disord 2005 Dec;89(1-3):125-35.
79. Clark L, Sarna A, Goodwin GM. Impairment of executive function but not memory in first-degree relatives of patients with bipolar I disorder and in euthymic patients with unipolar depression. American Journal of Psychiatry 2005;162(10): 1980-1982:-1982.
80. Smith DJ, Muir WJ, Blackwood DHR. Neurocognitive impairment in euthymic young adults with bipolar spectrum disorder and recurrent major depressive disorder. Bipolar Disorders 2006;8(1): 40-46:-46.
81. Nakano Y, Baba H, Maeshima H, Kitajima A, Sakai Y, Baba K, et al. Executive dysfunction in medicated, remitted state of major depression. J Affect Disord 2008 Nov;111(1):46-51.
82. Li CT, Lin CP, Chou KH, Chen IY, Hsieh JC, Wu CL, et al. Structural and cognitive deficits in remitting and non-remitting recurrent depression: a voxel-based morphometric study. NeuroImage 2010 Mar;50(1):347-56.
83. Biringer E, Lundervold A, Stordal K, Mykletun A, Egeland J, Bottlender R, et al. Executive function improvement upon

remission of recurrent unipolar depression. Germany: Springer; 2005.
84. Gallagher P, Robinson L, Gray J, Young A, Porter R. Neurocognitive function following remission in major depressive disorder: Potential objective marker of response? Australian and New Zealand Journal of Psychiatry 2007;41(1): 54-61:-61.
85. Christensen MV, Kyvik KO, Kessing LV. Cognitive function in unaffected twins discordant for affective disorder. Psychol Med 2006 Aug;36(8):1119-29.
86. Trichard C, Martinot JL, Alagille M, Masure MC, Hardy P, Ginestet D, et al. Time course of prefrontal lobe dysfunction in severely depressed in-p atients: a longitudinal neuropsychological study. Psychol Med 1995 Jan;25(1):79-85.
87. Beblo T, Baumann B, Bogerts B, Wallesch CW, Herrmann M. Neuropsychological Correlates of Major Depression: A Short-term Follow-up. Cognit Neuropsychiatry 1999;4(4):333-41.
88. Reppermund S, Ising M, Lucae S, Zihl J. Cognitive impairment in unipolar depression is persistent and non-specific: further evidence for the final common pathway disorder hypothesis. Psychol Med 2009 Apr;39(4):603-14.
89. Yuan Y, Zhang Z, Bai F, Yu H, You J, Shi Y, et al. Larger regional white matter volume is associated with executive function deficit in remitted geriatric depression: an optimized voxel-based morphometry study. J Affect Disord 2009 May;115(1-2):225-9.
90. Preiss M, Kucerova H, Lukavsky J, Stepankova H, Sos P, Kawaciukova R. Cognitive deficits in the euthymic phase of unipolar depression. Psychiatry Res 2009 Oct 30;169(3):235-9.
91. Rocca P, Calvarese P, Faggiano F, Marchiaro L, Mathis F, Rivoira E, et al. Citalopram versus sertraline in late-life nonmajor clinically significant depression: a 1-year follow-up clinical trial. J Clin Psychiatry 2005 Mar;66(3):360-9.
92. Alexopoulos GS, Meyers BS, Young RC, Campbell S, Silbersweig D, Charlson M. 'Vascular depression' hypothesis. Arch Gen Psychiatry 1997 Oct;54(10):915-22.
93. Lloyd AJ, Ferrier IN, Barber R, Gholkar A, Young AH, O'Brien JT. Hippocampal volume change in depression: late- and early-onset illness compared. Br J Psychiatry 2004 Jun;184:488-95.
94. Herrmann LL, Le MM, Ebmeier KP. White matter hyperintensities in late life depression: a systematic review. J Neurol Neurosurg Psychiatry 2008 Jun;79(6):619-24.
95. Austin MP, Mitchell P, Goodwin GM. Cognitive deficits in depression: possible implications for functional neuropathology. Br J Psychiatry 2001 Mar;178:200-6.
96. Austin MP, Mitchell P, Wilhelm K, Parker G, Hickie I, Brodaty H, et al. Cognitive function in depression: a distinct pattern of frontal impairment in melancholia? Psychol Med 1999 Jan;29(1):73-85.
97. MacDonald AW, III, Cohen JD, Stenger VA, Carter CS. Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control. Science 2000 Jun 9;288(5472):1835-8.
98. Ferrier IN, Thompson JM. Cognitive impairment in bipolar affective disorder: implications for the bipolar diathesis. Br J Psychiatry 2002 Apr;180:293-5.
99. Monteleone P, Serritella C, Martiadis V, Maj M. Decreased levels of serum brain-derived neurotrophic factor in both depressed and euthymic patients with unipolar depression and in euthymic patients with bipolar I and II disorders. Bipolar Disord 2008 Feb;10(1):95-100.
100. Molendijk ML, Bus BA, Spinhoven P, Penninx BW, Kenis G, Prickaerts J, et al. Serum levels of brain-derived neurotrophic factor in major depressive disorder: state-trait issues, clinical features and pharmacological treatment. Mol Psychiatry 2010 Sep 21.
101. Neumeister A, Yuan P, Young TA, Bonne O, Luckenbaugh DA, Charney DS, et al. Effects of tryptophan depletion on serum levels of brain-derived neurotrophic factor in unmedicated patients with remitted depression and healthy subjects. Am J Psychiatry 2005 Apr;162(4):805-7.
102. Kauer-Sant'anna M, Kapczinski F, Andreazza AC, Bond DJ, Lam RW, Young LT, et al. Brain-derived neurotrophic factor and inflammatory markers in patients with early- vs. late-stage bipolar disorder. Int J Neuropsychopharmacol 2009 May;12(4):447-58.
103. Kapczinski F, Vieta E, Andreazza AC, Frey BN, Gomes FA, Tramontina J, et al. Allostatic load in bipolar disorder: implications for pathophysiology and treatment. Neurosci Biobehav Rev 2008;32(4):675-92.
104. Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. Biol Psychiatry 2006 Jun 15;59(12):1116-27.
105. Dias VV, Brissos S, Frey BN, Andreazza AC, Cardoso C, Kapczinski F. Cognitive function and serum levels of brain-derived neurotrophic factor in patients with bipolar disorder. Bipolar Disord 2009 Sep;11(6):663-71.
106. Klein AB, Williamson R, Santini MA, Clemmensen C, Ettrup A, Rios M, et al. Blood BDNF concentrations reflect brain-tissue BDNF levels across species. Int J Neuropsychopharmacol 2010 Jul 7;1-7.
107. Kessing L. Validity of diagnoses and other clinical register data in patients with affective disorder. Eur Psychiatry 1998 Dec;13(8):392-8.
108. Bock C, Bukh JD, Vinberg M, Gether U, Kessing LV. Validity of the diagnosis of a single depressive episode in a case register. Clin Pract Epidemol Ment Health 2009;5:4.
109. Akiskal HS, Maser JD, Zeller PJ, Endicott J, Coryell W, Keller M, et al. Switching from 'unipolar' to bipolar II. An 11-year prospective study of clinical and temperamental predictors in 559 patients. Arch Gen Psychiatry 1995 Feb;52(2):114-23.
110. Zammit S, Allebeck P, David AS, Dalman C, Hemmingsson T, Lundberg I, et al. A longitudinal study of premorbid IQ Score and risk of developing schizophrenia, bipolar disorder, severe depression, and other nonaffective psychoses. Arch Gen Psychiatry 2004 Apr;61(4):354-60.
111. Miller GA, Chapman JP. Misunderstanding analysis of covariance. J Abnorm Psychol 2001 Feb;110(1):40-8.
112. Paykel ES. Partial remission, residual symptoms, and relapse in depression. Dialogues Clin Neurosci 2008;10(4):431-7.
113. Christensen MV, Kyvik KO, Kessing LV. Subclinical psychopathology and socio-economic status in unaffected twins discordant for affective disorder. J Psychiatr Res 2007 Apr;41(3-4):229-38.
114. The Danish National Board of Health. Referenceprogram for unipolar depression (Guidelines for treatment of unipolar disorder). http://www sst dk 2007.
115. Goswami U, Sharma A, Varma A, Gulrajani C, Ferrier IN, Young AH, et al. The neurocognitive performance of drug-free and medicated euthymic bipolar patients do not differ. Acta Psychiatr Scand 2009 Dec;120(6):456-63.

116. Goldberg JF, Chengappa KN. Identifying and treating cognitive impairment in bipolar disorder. Bipolar Disord 2009 Jun;11 Suppl 2:123-37.
117. Sheline YI, Gado MH, Kraemer HC. Untreated depression and hippocampal volume loss. Am J Psychiatry 2003 Aug;160(8):1516-8.
118. Pittenger C, Duman RS. Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology 2008 Jan;33(1):88-109.
119. Kessing LV, Sondergard L, Forman JL, Andersen PK. Antidepressants and dementia. J Affect Disord 2009 Sep;117(1-2):24-9.
120. Kessing LV, Forman JL, Andersen PK. Does lithium protect against dementia? Bipolar Disord 2010 Feb;12(1):87-94.
121. Kent S, Yellowlees P. Psychiatric and social reasons for frequent rehospitalization. Hosp Community Psychiatry 1994 Apr;45(4):347-50.
122. Middeldorp CM, Cath DC, van DR, Boomsma DI. The co-morbidity of anxiety and depression in the perspective of genetic epidemiology. A review of twin and family studies. Psychol Med 2005 May;35(5):611-24.