A longitudinal investigation of cortical plasticity and structure in bipolar disorder type II

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# Table of contents

Acknowledgements ..................................................................................................................... 4  
List of papers............................................................................................................................... 7  
Summary...................................................................................................................................... 8  
Abbreviations ............................................................................................................................ 10  
1. Introduction........................................................................................................................... 12  
   1.1 Overview ..................................................................................................................... 12  
   1.2 Bipolar disorder type II................................................................................................. 13  
   1.3 Synaptic plasticity and bipolar disorder ........................................................................ 20  
   1.4 Methods for examinations of cortical plasticity and thickness ....................................... 25  
2. Aims of the thesis .................................................................................................................. 30  
3. Materials and methods ......................................................................................................... 31  
   3.1 Setting .......................................................................................................................... 31  
   3.2 Study participants .......................................................................................................... 31  
   3.3 Clinical assessments ....................................................................................................... 34  
   3.4 VEP plasticity ................................................................................................................ 36  
   3.5 Saliva collection and cortisol analysis .......................................................................... 37  
   3.6 MRI acquisition and analysis ...................................................................................... 37  
   3.7 Statistical analysis ........................................................................................................ 38  
   3.8 Ethical considerations .................................................................................................... 39  
4. Summary of papers ............................................................................................................... 40  
   4.1 Paper I: Longitudinal and Cross-Sectional Investigations of Long-Term Potentiation- 
      Like Cortical Plasticity in Bipolar Disorder Type II and Healthy Individuals .............. 40  
   4.2 Paper II: Mood Episodes are Associated With Increased Cortical Thinning: A 
      Longitudinal Study of Bipolar Disorder Type II ............................................................... 41  
   4.3 Paper III: Long-Term Potentiation-Like Cortical Plasticity is Associated With Cortical 
      Thinning in Healthy Individuals and Bipolar Disorder: A Longitudinal Study ............. 42  
5. Discussion .............................................................................................................................. 43  
   5.1 Discussion of main findings ........................................................................................... 43  
   5.2 Methodological considerations ..................................................................................... 55  
   5.3 Summary and implications for future research ............................................................. 59  
6. Concluding thoughts............................................................................................................. 62  
7. References .............................................................................................................................. 63
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List of papers

Paper I:

Paper II:
Zak N, Bøen E, Boye B, Andreassen OA, Doan NT, Malt UF, Westlye LT, Elvsåshagen T. Mood episodes are associated with increased cortical thinning: a longitudinal study of bipolar disorder type II. In revision.

Paper III:
*Shared first authorship.
Summary

Background

Bipolar disorder (BD) type II is characterized by episodes of elated and depressed mood and energy, and can cause substantial social and occupational impairments. The neurobiological substrates of the disorder remain unknown and identification of central disease mechanisms is essential for developing more effective therapeutic strategies and improving patient outcomes. BDs have in recent years been conceptualized as genetically influenced disorders of synaptic function and plasticity, resulting in destabilization and loss of synapses in mood and emotion circuitry. Previous cross-sectional studies found evidence for impairments in cortical long-term potentiation (LTP)-like plasticity and reduced frontotemporal cortical thickness in individuals with BD type II compared to healthy controls (HCs). However, whether these changes represent stable traits predisposing to illness or effects of mood episodes remains to be clarified.

Aims

The overall aim of the present thesis was to contribute to greater understanding of cortical alterations and their longitudinal trajectories in BD type II. Our research group previously published cross-sectional evidence for impaired plasticity of the visual evoked potential (VEP), i.e., a promising assay for non-invasive studies of LTP-like cortical plasticity, and for reduced frontotemporal cortical thickness in patients with BD type II. The present thesis examined the reproducibility of these findings, assessed whether they are stable disease traits or progress over the illness course, and investigated the relationship between cortical changes and mood episodes. Based on the hypothesis that loss of dysfunctional synapses might contribute to cortical thinning in BDs and in normal aging, we examined the association between VEP plasticity and longitudinal cortical thickness changes.

Material and methods

Adults with BD type II and HCs underwent examinations of VEP plasticity, magnetic resonance imaging (MRI), and clinical interviews at baseline, and on average 2.4 years later, at follow-up. To examine VEP plasticity, participants underwent a checkerboard reversal paradigm during recording of the electroencephalogram (EEG). Visual stimuli were presented with E-Prime 1.1 and EEG analysis was conducted using EEGLAB. Structural MRI data was acquired using a 3T Philips Achieva Scanner and analyzed with Freesurfer 5.3.
Main findings

Patients had reduced VEP plasticity compared to HCs at follow-up, consistent with previously published baseline results. Moreover, VEP plasticity was impaired in euthymic patients, was negatively associated with severity of depressive symptoms, and remained significantly reduced in patients after covarying the analyses for saliva cortisol. Saliva cortisol was positively correlated with VEP plasticity in HCs, but not in patients. Plasticity of the P1 and N1 VEP amplitudes in HCs and plasticity of the P1-N1 amplitude in patients showed moderate temporal stability when results from baseline and follow-up examinations were compared.

MRI-based measurements showed that patients had thinner cortices in bilateral frontotemporal regions at follow-up, consistent with baseline results. Longitudinal analyses showed widespread cortical thinning in both groups, however, patients had increased rate of cortical thinning in a left temporal region relative to HCs. Furthermore, larger number of depressive episodes between baseline and follow-up examinations was associated with a higher rate of thinning in the left temporal cortex and in ventromedial prefrontal cortices, although the latter result did not survive correction for multiple analyses.

Plasticity of the N1 VEP amplitude at baseline was negatively associated with thinning in widespread cortical regions across healthy individuals and patients, and in healthy individuals alone. Plasticity of the P1 amplitude was positively associated with cortical thinning in the total sample; this finding was not significant after removing outliers from the analysis.

Interpretation and implications

The results of the present thesis support recent models of BDs, which suggest that impaired synaptic function and plasticity in mood and emotion circuitries are central pathophysiological mechanisms. The findings indicate that impaired VEP plasticity and reduced frontotemporal cortical thickness are stable traits in adults with BD type II, and that mood episodes might cause further deterioration of these cortical abnormalities. Finally, the results point to an association between VEP plasticity and longitudinal changes in cortical thickness in patients with BD type II and in healthy individuals. Future studies are needed to clarify whether genetic risk variants for BD type II affect VEP plasticity and cortical thickness, whether treatments can reverse the cortical alterations, and whether the observed changes are specific for BD type II or common neurobiological characteristics of mood disorders.
**Abbreviations**

ACC – anterior cingulate cortex

AEP – auditory evoked potential

AN(C)OVA – analyses of (co)variance

BD – bipolar disorder

BDNF – brain-derived neurotrophic factor

DSM – Diagnostic and Statistical Manual of Mental Disorders

dlPFC – dorsolateral prefrontal cortex

ECT – electroconvulsive treatment

EEG – electroencephalography

ERP – evoked response potential

fMRI – functional magnetic resonance imaging

GABA – gamma-amino-butyric acid

GMV – gray matter volume

GWAS – genome-wide association studies

HCs – healthy controls

HPA axis – hypothalamic-pituitary-adrenal axis

LTP – long-term potentiation

MADRS – Mongomery-Aasberg Depression Rating Scale

MDD – major depressive disorder

MRI – magnetic resonance imaging

NMDA – N-methyl-D-aspartate

PAS – paired associative stimulation
PFC – prefrontal cortex

ROI – region of interest

SNP – single nucleotide polymorphism

SWA – slow wave activity

tDCS – transcranial direct current stimulation

TMS – transcranial magnetic stimulation

VBM – voxel-based morphometry

VEP – visual evoked potential

vIPFC – ventrolateral prefrontal cortex

V1 – primary visual cortex

YMRS – Young Mania Rating Scale
1. Introduction

1.1 Overview

Alternating episodes of elevated and depressed mood occurring in the same individual are reported across time and cultures [1]. Ancient Greek physicians first described “mania” and “melancholia”, and suggested they might be two different states of the same disease [2]. The current diagnostic construct of bipolar disorders (BDs) is based on clinical observations made by 19th century psychiatrists and supported by research in the past decades [2]. In 1994, the fourth edition of the American Psychiatric Association’s *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) drew a distinction between two main subtypes of BD: BD type I, which is defined by a lifetime episode of mania, and BD type II, which is characterized by episodes of hypomania and depression [3]. Diagnoses of BDs are based on self-report and observations of behavior and the precise pathophysiological mechanisms remain elusive [4]. Current treatments are therefore symptomatic and may not target fundamental disease processes [5]. Increased insight into central underlying mechanisms is a critical first step towards improved diagnostic tools, more effective treatments, and better outcomes in BD type I and II.

Hippocrates (460-377 BC) theorized that mental functions and disturbances of these originated in the brain, however, scientific search for lesions underlying neuropsychiatric illnesses first began two centuries ago with the German psychiatrist Griesinger’s maxim that mental illnesses are brain illnesses [6]. The following search for pathophysiological mechanisms was hampered by lack of non-invasive methods for studying the human brain. Recent advancements in neuroimaging techniques have therefore been instrumental in identifying brain regions implicated in BD pathophysiology, and indicate that BDs are associated with structural and functional abnormalities in brain circuits supporting emotion and reward processing and mood regulation [7-9]. Moreover, results from preclinical and clinical studies suggest that neural dysfunction in these brain networks may result from genetically influenced dysregulation of synaptic function and plasticity [10-18]. Consistent with these findings, previous studies found evidence for impaired cortical plasticity and reduced cortical thickness in brain regions involved in mood regulation in BD type II [19-21]. Whether these cortical abnormalities represent neurodevelopmental traits predisposing to illness or an effect of mood episodes remains unknown.
BD type I and II are associated with marked social and occupational disability and are major contributors to the global burden of disease [22]. BD type II might be more prevalent than BD type I and the clinical significance of these subtypes are comparable in terms of illness burden, role impairments, and suicide attempts [23]. Although it is unknown whether mania and hypomania are associated with different pathophysiological mechanisms, there are several lines of evidence suggesting genetic and neurobiological differences between individuals with BD type I and BD type II [24-31]. In light of these findings, the pathophysiology of BD type II remains markedly understudied. The main objective of this study was therefore to increase our understanding of structural and functional abnormalities in the cerebral cortex and their longitudinal trajectories in BD type II.

Chapter 1 of this thesis will give an overview of BD type II, including diagnostic criteria, epidemiology, clinical features and treatment options, and current understanding of etiology and pathophysiology. The next sections will introduce synaptic plasticity, discuss evidence for plasticity impairments in BDs and other mood disorders, and then present current methods for non-invasive examination of cortical plasticity and thickness in humans.

Chapter 2 describes the aims of this study, while Chapter 3 presents the materials and methods. The main findings of the three papers included in this thesis are summarized in Chapter 4, the findings and their implications for future research are discussed in Chapter 5, and the concluding thoughts of Chapter 6 end the thesis.

1.2 Bipolar disorder type II

The current knowledge of BD type II will be summarized in this chapter and includes diagnostic criteria (Section 1.2.1), epidemiology, clinical features, and treatment (Section 1.2.2), and etiology and pathophysiology (Section 1.2.3).

1.2.1 Diagnostic criteria

The diagnosis of BD type II is based on symptoms, signs, and illness course, and there are no objective biological measures for confirming the diagnosis. Table 1 on page 14 shows the diagnostic criteria for BD type II according to the DSM-IV [32], which were used for patient inclusion in the present study. Here, BD type II is defined by at least one lifetime episode of hypomania and at least one lifetime major depressive episode.

A hypomanic episode is defined as a distinct period of persistently elevated, expansive, or irritable mood, lasting throughout a minimum of four days. It must be clearly different from
non-depressed mood, associated with a change in functioning that is uncharacteristic of the person, and is observable by others. The episode is not severe enough to cause marked impairments in social or occupational functioning or to require hospitalization, and psychotic symptoms cannot be present. The symptoms cannot be caused by substance use or a medical condition. In addition, at least three of the following symptoms (four if the mood is only irritable) have to be present to a significant degree: inflated self-esteem or grandiosity, decreased need for sleep, more talkative than usual or pressure to keep talking, flight of ideas or racing thoughts, distractibility, increase in goal-directed activity or psychomotor agitation, and excessive involvement in pleasurable activities that have potential painful consequences.

A major depressive episode is defined as a period of at least two weeks where depressive symptoms have to be present most of the day nearly every day, represents a change from previous functioning, and cause significant distress or impairments in social or occupational functioning. At least five of the following symptoms must be present: depressed or irritable mood, diminished interest or pleasure in most or all activities, change in weight or appetite, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness or excessive guilt, diminished ability to think or concentrate or indecisiveness, and suicidality. At least one of the symptoms has to be either depressed mood or loss of interest or pleasure, symptoms must not meet criteria for a mixed episode, and cannot be caused by substance use, a medical condition, mood-incongruent delusions or hallucinations, or be better accounted for by bereavement.

<table>
<thead>
<tr>
<th>Table 1. DSM-IV-TRa diagnostic criteria for bipolar disorder type II</th>
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<tbody>
<tr>
<td>A. Presence (or history) of one or more major depressive episode</td>
</tr>
<tr>
<td>B. Presence (or history) of at least one hypomanic episode</td>
</tr>
<tr>
<td>C. There has never been a manic episode or a mixed episode</td>
</tr>
<tr>
<td>D. The mood symptoms in Criteria A and B are not better accounted for by schizoaffective disorder and are not superimposed on schizophrenia, schizophreniform disorder, delusional disorder, or psychotic disorder not otherwise specified</td>
</tr>
<tr>
<td>E. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning</td>
</tr>
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</table>

*aDiagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision [32]*
Bipolar disorder in DSM-V
The 5th edition of the DSM was published in 2013 [33] and for the first time, bipolar and related disorders were considered separate from depressive disorders. Furthermore, the definition of a hypomanic episode was revised so that persistently increased activity or energy levels must accompany the mood change. Moreover, the number of exclusion criteria was reduced; for instance, recent bereavement does no longer exclude the diagnosis of a major depressive episode, and antidepressant treatment does no longer exclude a hypomanic episode. Other changes include the possibility to specify BD with mixed features. Lastly, subthreshold syndromes were unified under the NOS (not otherwise specified) heading.

1.2.2 Epidemiology, clinical features, and treatment

Epidemiology
Estimates of lifetime prevalence of BD type II vary across time and sites, and are influenced by diagnostic criteria, particularly the minimum duration criteria. A review of historical studies concluded that “a reasonable overall figure” for BD type II is “in the vicinity of 5%” [34]. Another review reported a prevalence rate of 0.4% when diagnostic criteria where based on the DSM-IV [23], yet the rate of sub-threshold BD was 1.4%, and a substantial number of these individuals probably met the criteria for BD type II except for the duration criteria [23]. These findings suggest that BD type II might be the most prevalent subtype [35], and that a broader definition of hypomania significantly increases prevalence rates [36-38]. In addition, it has been suggested that rates of BD type II are increasing; it is not known whether this observation reflects a true increase in prevalence or a greater number of individuals receiving correct diagnosis [39]. Patients usually experience their first mood episode in their early twenties [23, 40], yet years may pass before a correct diagnose is received since BD type II cannot be differentiated from major depressive disorder (MDD) until the first episode of hypomania [36, 41, 42]. Furthermore, patients and clinicians often overlook hypomanic symptoms, and a substantial number of patients are therefore incorrectly diagnosed with and treated for MDD [43].

Clinical features
“From the time I woke up in the morning until the time I went to bed at night, I was unbearably miserable and seemingly incapable of any kind of joy or enthusiasm. Everything—every thought, word, movement—was an effort. Everything that once was sparkling now was flat.” [44].

15
Depression is the most common clinical presentation of BD type II and dominates the longitudinal course [45-47]. Prospective follow-up studies indicate that patients with BD type II are euthymic approximately half of the time, depressed or experience depressive symptoms almost half of the time, and have hypomanic symptoms only a few percent of the time [45, 48]. Sadness, apathy, anxiety, or irritability are core mood disturbances, and often lead to social and occupational impairments [22]. On a group level, patients with BD type II experience psychosocial impairment during most of the illness course, and are unable to carry out work role functions during approximately 20% of the time [49]. About 1/3 of patients will attempt suicide [50, 51], usually during a depressive episode [52], and the risk for completed suicide is 20-30 times higher than for the general population [53]. However, the severity of the illness trajectory is heterogeneous and some patients experience few mood episodes and have complete functional recovery, whereas others have a progressive illness course with shortening of inter-episode intervals and impaired response to treatment [54, 55].

Comorbidity is highly prevalent in BD type II and recent epidemiological surveys found that approximately 85% of the patients had a history of three or more DSM-IV disorders, most commonly anxiety disorders, such as panic disorder, general anxiety disorder, and social phobia [23, 40]. Furthermore, substance abuse was found in over 40% of individuals with BD type II, with alcohol being the most common agent of misuse [40]. In addition, impairments are found in all cognitive domains, even after long periods of euthymia [56], and cognitive decrements contribute to unfavorable functional outcomes [57].

Treatment

There is a scarcity of randomized placebo-controlled treatment trials in BDs in general and BD type II in particular, and national guidelines differ in whether or not they include separate recommendations for the two subtypes [58]. Acute hypomania in BD type II is often self-limiting, however, treatment is recommended for prolonged episodes. Some guidelines provide specific treatment recommendations for hypomania, usually the same treatment as for mania, i.e., monotherapy with a mood stabilizer or an antipsychotic agent [58]. Hypomania should according to Norwegian guidelines be treated with limitation of activity levels, protection from overstimulation, a regular circadian rhythm, and acute treatment with valproate or quetiapine, if necessary [59]. However, since hypomania does not lead to significant dysfunction, the main focus in BD type II is treatment of acute depression and prevention of future depressive episodes [60].
A review of pharmacological treatments for acute depression in BD type II concluded that there is empirical support from randomized trials for quetiapine monotherapy [61]. Quetiapine monotherapy is also the recommended treatment choice in the Norwegian guidelines [59], and the only drug approved by the US Food and Drug Administration (FDA) for treatment of depression in BD type II [62]. There is also preliminary evidence for lithium, antidepressants, and pramipexole in the treatment of depressive episodes of BD type II [61]. Norwegian guidelines recommend a combination of olanzapine and fluoxetine, and lamotrigine or lithium as second line options [59]. In general, antidepressants in monotherapy are not used in bipolar depression since they may induce a switch to mania or a destabilization of the illness in the absence of a concurrent mood stabilizer [63, 64]. This underscores the importance of receiving a correct diagnosis as many patients initially are misdiagnosed and treated for MDD. However, there are studies suggesting that antidepressants in monotherapy may be appropriate for some patients with BD type II [65, 66].

Electroconvulsive therapy (ECT) is recommended as third-line treatment for acute depressive episodes in BD type II [58], also in Norwegian guidelines [59].

Maintenance treatment after remission of a mood episode is challenging, but important for preventing progression and relapse, especially if depressive episodes are severe and the inter-episode interval begins to shorten. Maintenance treatment in BD type II focuses on prevention of depressive episodes [67], and lamotrigine or quetiapine are most commonly recommended as medications of choice in guidelines with specific recommendations for BD type II [58]. Norwegian guidelines recommend lamotrigine as first line treatment, and lithium, quetiapine, valproate, or antidepressants as second line treatments [59]. Furthermore, although the evidence for specific adjunctive psychotherapies in BD type II is limited, Norwegian guidelines recommend individual or group psychoeducation [59].

Approximately 60-80% of patients experience recurrence of mood episodes after discontinuation of treatment, and 20-50% relapse during ongoing therapy [68], indicating that current treatment options may not target key pathophysiological mechanisms. In addition, current antidepressants require several weeks to take effect [69]. Thus, there is a clear need for more effective and faster acting treatment options for bipolar depression. The rapid-acting N-methyl-D-aspartate (NMDA)-receptor antagonist ketamine is a potential candidate and has shown short-term effectiveness after a single intravenous administration in several controlled trials in patients with BD type I and type II [70, 71].
Treatment-resistant bipolar depression remains a therapeutic challenge, and neuromodulatory treatments are emerging as promising options. There is a paucity of studies investigating treatment effects of neuromodulation in “pure” BD type II samples, yet multiple studies have included mixed groups of individuals with BD type I and II. During transcranial magnetic stimulation (TMS), a series of pulsed magnetic stimuli are administered using a stimulating coil applied directly to the head. This stimulation induces an electrical current in the underlying cortical tissue and causes depolarization of neurons and subsequent change in cortical neuronal function [72]. TMS is becoming an accepted treatment for MDD, and its effectiveness in the BDs is promising, albeit less studied [73, 74]. Transcranial direct current stimulation (tDCS) refers to application of a weak direct current via scalp electrodes overlying targeted cortical areas, which induces changes in the neural membranes between the electrodes [75]. It has been hypothesized that tDCS facilitates synaptic plasticity through long-term potentiation (LTP)-like mechanisms [76]. A recent review and meta-analysis concludes that tDCS significantly improves depressive symptoms in subjects with BD, however, more research on safety and clinical effects are needed before it can be implemented in daily clinical practice [77]. Vagus nerve stimulation (VNS) indirectly modulates brain network activity through stimulation of a cranial nerve, and can be performed through implantation of a small pulse generator, or non-invasively through transcutaneous application [78]. It is assumed that VNS acts via innervation of sensory nuclei in the brain stem and modulates projections to limbic brain regions and other frontal cortical areas, yet the precise mechanisms remain to be elucidated [79]. Although studies in BDs are sparse, limited evidence suggests a beneficial effect of VNS on treatment-resistant depression in BD type I and II [78]. The use of deep brain stimulation (DBS) has emerged as an additional therapeutic option for treatment resistant depression [80]. Different sites for stimulation have been investigated, including the subcallosal cingulate, the ventral capsule/ventral striatum, the nucleus accumbens, the lateral habenula, the inferior thalamic peduncle, and the medial forebrain bundle (which interconnects all previous targets) [81]. The precise mechanisms for antidepressant effects of DBS remain unclear, yet may include acute modulatory effects of emotion circuitry causing immediate mood improvement and long-term neural reorganization leading to lasting clinical improvement and remission in some patients [82, 83]. A few studies have examined the effect of DBS on depressive symptoms in BD type II, and the results are promising [81, 84].
1.2.3 Etiology and pathophysiology

The etiology and pathophysiology of BD type II are understudied and remain to be clarified. This section will present a brief overview of biological pathways that may be implicated in BD type II and summarize the findings from neurobiological studies. The potential roles of synaptic function and plasticity in the pathophysiology of BD type II will be discussed in Section 1.3 in more detail.

Biological pathways implicated in BD type II

The etiology of BD type II remains unclear, yet the heritability of around 0.6 suggests involvement of a strong genetic component [85, 86]. No single gene has been implicated in BD type II, and numerous genetic variants with small effects may together result in disease vulnerability, possibly through interaction with environmental risk factors [87, 88]. Genome-wide association studies (GWAS) that included mixed samples of BD type I and II have identified risk loci linked to several biological pathways, including immune signaling, histone methylation (implicating epigenetic regulatory mechanisms), corticotropin-releasing hormone signaling (implicating the stress-responsive hypothalamic-pituitary-adrenal (HPA)-axis), synaptic calcium- and glutamate signaling, and synaptic plasticity [89-91]. There is a paucity of studies focusing on BD type II, yet limited data suggest that variants of genes involved in synaptic signal transduction, neurodevelopmental function, immune and inflammatory response [86], and circadian genes [92] are associated with increased illness risk. The involvement of these biological pathways is supported by studies showing that BD type II is associated with elevated levels of oxidative stress markers [93], with increased load of short telomeres, which may represent accelerated aging [94], with increased levels of cytokines, indicating inflammatory dysregulation [95], and with circadian rhythm dysregulation [92, 96]. Furthermore, altered concentrations of cerebrospinal fluid markers for synaptic function have been reported in euthymic patients with BD type II [97].

Neurobiological alterations in BD type II

Neuroimaging studies indicate that patients with BD type II show reduced cortical thickness in prefrontal, temporal, and cingulate regions [9, 21]. These findings are in line with postmortem observations of reduced neuronal and glial cell size and density in prefrontal and anterior cingulate cortices in BDs (although there is a lack of histological studies focusing on BD type II) [98-100]. Furthermore, a recent cross-sectional study suggested thinner prefrontal cortices in patients with higher lifetime number of depressive episodes [101]. Other brain changes
reported in BD type II are abnormalities in frontal white matter volume [102] and density [103], possibly affecting tracts connecting frontal regions with subcortical structures [104]. Functional MRI (fMRI) studies have revealed reduced frontal, temporal, and cerebellar interhemispheric connectivity in patients with BD type II [105-107]. Furthermore, reduced functional connectivity between the right amygdala and right prefrontal cortices, and reduced activation in prefrontal and temporal regions during emotional processing have been reported in depressed patients with BD type II [108]. However, other fMRI studies did not find deficits related to impulsivity [109] and emotion regulation [110] in individuals with BD type II. Metabolic abnormalities are suggested by increased levels of the amino acids glutamate and glutamine (Glx) in gray and white matter [111], and by reduced levels of chemicals related to cellular energy and phospholipid metabolism in left dorsolateral prefrontal cortex (dlPFC) [112], in unmedicated patients with BD type II. Reduced ratio of N-acetyl aspartate (NAA)/creatine-phosphocreatine in left dlPFC of patients with BD type II might represent decreased neuronal density or neuronal dysfunction [113].

Although the number of neuroimaging studies conducted to date is limited, these findings implicate abnormalities in function and structure of brain regions important for mood regulation in the pathophysiology of BD type II. However, longitudinal studies are warranted to clarify whether the findings represent developmental abnormalities predisposing to illness or effects of mood episodes. Furthermore, the precise cellular processes underlying these alterations remain elusive, yet dysregulated synaptic function and plasticity are two of the leading candidate mechanisms [15, 114-116].

1.3 Synaptic plasticity and bipolar disorders

Synapses are highly specialized structures that allow one neuron (the presynaptic neuron) to communicate with the next (the postsynaptic neuron) using neurotransmitters, as shown in Figure 1A on page 21 [117]. Neural circuits in the brain are established and maintained via synaptic connections, allowing for targeted and efficient information transfer. Signal transmission across synapses can be modified through activity-dependent and homeostatic processes, which are essential for brain development, information processing, learning and memory [118]. Synaptic plasticity refers to the capacity of synapses for functional and structural changes and can involve both presynaptic and postsynaptic mechanisms [119]. The best-characterized form of synaptic plasticity is LTP [120], which will be introduced in Section 1.3.1. A growing body of evidence indicates that maladaptive synaptic plasticity may contribute to
neuropsychiatric disorders [11, 15, 118], and evidence for impaired synaptic plasticity in BDs will be reviewed in Section 1.3.2.

1.3.1 Synaptic plasticity and long-term potentiation

One of the most influential theories in modern neuroscience, proposed by Donald Hebb in 1949, predicted that learning and memory would involve an increase in synaptic efficacy elicited by coordinated pre- and post-synaptic firing [121]. Synaptic plasticity is an important aspect of most current models of learning and behavioral changes [122-125], and the best-characterized form is LTP of excitatory synaptic transmission, as illustrated in Figure 1B [126-128]. This phenomenon was first described by Lømo and Bliss in 1973 when they showed that a burst of tetanic stimulation of the presynaptic neuron resulted in a long-lasting augmentation of the postsynaptic response in the rabbit hippocampus [129].

![Figure 1](image)

**Figure 1.** (A) shows a synapse with an axonal bouton (presynaptic neuron), the synaptic cleft, a neurotransmitter, and receptors at the dendritic spine (postsynaptic neuron). (B) illustrates long-term potentiation (LTP) of synaptic transmission after repeated stimulation, with enlargement of the axonal bouton and dendritic spine and increased postsynaptic potential. The figures were made by Zak A.

LTP describes a family of processes that strengthen synaptic transmission based on recent patterns of synaptic activity through increased efficiency and number of glutamatergic alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors [126]. LTP is characterized by distinct cellular and molecular mechanisms: it is dependent on the postsynaptic NMDA type of glutamate receptor and exhibit several defining properties that are required for information storage [130]. LTP is *input specific* since it is induced by synaptic activity in the pathway that is being potentiated, and does not spread to synapses that are not stimulated [131].
A weak stimulation is normally not sufficient to induce LTP, however, if applied simultaneously to a strong stimulation of another pathway into a synapse, LTP will be induced in both pathways, which is referred to as associativity [131]. Furthermore, LTP can be induced by repeated strong stimulations of a single pathway into a synapse or by weak cooperative activations of separate but converging pathways [132]. This might generate sufficient depolarization of the post-synaptic cell to induce LTP in all pathways, and the mechanisms underlying associativity and cooperativity might be overlapping. Finally, LTP is characterized by persistence over time, and may last from minutes to many months [133]. Although the precise molecular processes responsible for stabilization and maintenance of LTP are not fully clarified, they are dependent on protein kinase Mζ [134, 135] and brain-derived neurotrophic factor (BDNF) [136].

Long-term modifications of synaptic efficacy are believed to take place in most synapses in the brain [126], including cortical synapses [137-140]. Furthermore, they are associated with structural changes; induction of LTP can increase the number of cortical synapses [141, 142], while long-term depression (LTD), i.e., an activity-dependent reduction in synaptic strength [143], is associated with synaptic loss [144-148]. Dysregulated synaptic plasticity, e.g., due to genetic vulnerability and environmental factors such as prolonged stress, might lead to destabilization and loss of synaptic connections in neural circuits, and has been proposed to underlie structural brain changes and pathological symptoms and behaviors in a number of neuropsychiatric conditions [149, 150].

1.3.2 Evidence for impaired synaptic plasticity in bipolar disorder

Dysregulated synaptic function and plasticity in mood and emotion circuitry are leading candidate mechanisms in mood disorders [11, 114]. Furthermore, normalization of synaptic plasticity and connectivity in these networks might be a neurobiological substrate for treatment effects [10, 116, 151, 152]. There is currently a scarcity of neurobiological studies in BD type II, yet converging lines of findings from postmortem studies, genetic studies, rodent models, studies of treatment targets in the BDs, and recent in vivo experiments in patients with BD type II together support these hypotheses [19, 99, 116, 153, 154].

There has been no histological post-mortem study focusing on BD type II, most post-mortem studies did not specify the subtype of BD and they might include mixed samples. The most consistently reported microstructural brain changes in BDs include decreased size and density of glia and neurons in prefrontal and anterior cingulate cortices [98, 99]. Astrocytes are involved
in formation and remodeling of synapses, and reduced glial density might be associated with decreased number of functional synapses in BDs [99]. Indeed, studies report altered expression of netrin-G1, netrin-G2, and growth-associated protein-43, which are markers of synaptic function and plasticity, in the ACC [155], temporal cortex [156], and hippocampus [157] of patients with BDs. Furthermore, altered levels of proteins important for synaptic glutamate release in the ACC [158], hippocampus [159], and the occipital cortex [160], and abnormal levels of proteins that regulate release of glutamate and GABA and synaptic plasticity in the cerebellum have been reported [161]. Moreover, increased protein and mRNA levels of the pro-apoptotic markers Bax, BAD, caspase-9 and caspase-3, reduced protein and mRNA levels of the anti-apoptotic markers BDNF and Bcl-2, and reduced levels of pre-synaptic (synaptophysin) and post-synaptic (drebrin) markers have been detected in prefrontal cortex of BDs patients compared to controls [162]. These post-mortem results together support synaptic dysfunction in BDs pathophysiology, and indicate that alterations are not confined to brain areas primarily involved in emotion processing and regulation.

GWAS indicate that BDs risk is associated with genetic variants important for synaptic function and plasticity, including voltage-gated calcium channels, glutamate receptor signaling, and LTP [89-91]. However, only one GWAS has exclusively examined patients with BD type II and found novel susceptible loci, but no overlap with genes reported in previous studies [86]. This study used pathway analyses to show that, among others, genes involved in synaptic signal transduction were significantly associated with BD type II [86]. The patients included in this study had less comorbidity compared to previous studies, perhaps reducing confounding effects from other genetic conditions. However, the sample size was modest, and the findings need to be replicated [86]. Nevertheless, these studies together suggest a genetic vulnerability for synaptic deficits in BD type II.

It is challenging to accurately model BD type II in animals given the subjective nature of several core symptoms, the cyclical nature of mood episodes, and the current lack of objective biomarkers [163, 164]. Nonetheless, animal models can provide insights into implicated molecular and cellular pathways. Current animal models mimicking facets of hypomania and depression support an association between BD phenotypes and changes in synaptic function and plasticity [11, 165]. For instance, mutations in SHANK3, a postsynaptic density protein involved in neurotransmission and synaptic formation, result in mania-like behaviors in rodents [166]. Furthermore, inhibition of ion transport across the cell membrane by the toxin ouabain is associated with mania-like behavior [167], decreased brain levels of BDNF, and abnormal
short- and long-term synaptic plasticity in the PFC of rodents [168]. One of the best established models of depression involve stress-induced changes in behavior [169] since stressful life events are important risk factors for depressive disorders [170]. Prenatal stress in rodents is associated with changes in the HPA-axis [171] and reduced BDNF levels and increased expression of glutamate transporters in the hippocampus and the PFC [172, 173]; these are all factors important for synaptic transmission and plasticity. Furthermore, in other stress-related models depressive-like behavior is associated with acute and persistent loss of synaptic spines and dendrites in the hippocampus and in prefrontal cortices, reduced hippocampal neurogenesis, and altered long-term synaptic plasticity [174-179].

Although the precise mechanisms of action for many current pharmacological therapies in BD type II are unclear [73], preclinical studies suggest they might normalize mania- and depression-like phenotypes by reversing synaptic impairments [180]. Lithium acts on a number of molecules within second messenger systems, which in turn modulate neurotransmission, reduce oxidative stress, and increase levels of neuroprotective proteins [181]. At a neuronal level, lithium acts both pre- and post-synaptically and inhibits excitatory synaptic activity, while facilitating inhibitory neurotransmission [181]. Lithium also has antidepressive properties, possibly through maintenance of hippocampal cell turnover and synaptic plasticity [182, 183]. Furthermore, both lithium and valproate regulate AMPA receptor trafficking by down-regulating synaptic expression of the GluR1 receptor subunit, which is important for synaptic activity regulation and possibly for functioning of the neuronal circuitry that support mood regulation [152, 184]. Atypical antipsychotic medications modulate dopamine-glutamate interactions at the post-synaptic density, potentially affecting dopamine and glutamate-mediated synaptic plasticity [185], and their long-term effects might be mediated by induction of postsynaptic remodeling [186]. Quetiapine may reduce stress-induced decreases in hippocampal neurogenesis and BDNF expression [187], and enhance BDNF release from astrocytes [188]. Moreover, studies suggest that antidepressants increase BDNF expression and modulate LTP in vitro [189-193] and in vivo [194-199]. A recent study reported that SSRI treatment inhibited stress-induced facilitation of LTD in hippocampal neurons by directly blocking voltage-activated L-type calcium channels [200]. Mechanisms related to LTP and LTD may also contribute to the antidepressant effects of ECT [199, 201, 202]. Furthermore, the discovery of rapid acting antidepressants, including ketamine, was based on research indicating that glutamatergic synaptic transmission was affected in depression [203, 204]. Ketamine is an NMDA receptor antagonist and may exert its rapid antidepressant effect by
inducing a glutamate burst through disinhibition of pyramidal neurons; this could depolarize postsynaptic cells, and, through a cascade of events, ultimately result in synaptogenesis and altered synaptic plasticity [151, 205-208].

Non-invasive electrophysiological indices can provide in vivo evidence for impairments in synaptic function and plasticity in patients. A previous study in BD type II found reduced amplitude of the auditory mismatch negativity (MMN) [209], which is an auditory electroencephalography (EEG) response to a deviant stimuli occurring in a stream of standard auditory stimuli [210]. MMN depends on intact NMDA receptor signaling, and is often used as a non-invasive index of glutamatergic synaptic function and short-term plasticity [210, 211]. Furthermore, previously published results from the baseline examination of the current study showed impaired cortical LTP-like plasticity in individuals with BD type II [19]. These findings provide in vivo evidence for synaptic function and plasticity impairments in this disorder. However, there is a scarcity of longitudinal LTP-like plasticity studies, and it remains largely unknown whether non-invasive indices of cortical synaptic plasticity [120, 212, 213] correlate with clinical variables.

1.4 Methods for examinations of cortical plasticity and thickness

Modern neurophysiological and neuroimaging techniques allow for examinations of cortical plasticity and structure non-invasively in humans. These methods may advance our understanding of neurobiological mechanisms underlying BD type II and other psychiatric illnesses.

1.4.1 Methods for in vivo measurement of cortical plasticity

The scalp EEG mainly reflects cortical postsynaptic potentials [214]. Repetitive sensory, electric, and magnetic stimulation can induce lasting changes in EEG-derived measures and these are increasingly used as non-invasive indices of cortical synaptic function and plasticity [120, 212, 213]. Current methods mainly encompass repetitive stimulation-induced plasticity in sensory and motor cortices [120, 215]. In sensory cortices, repetitive auditory and visual stimulation can induce LTP-like amplitude enhancement of the auditory evoked potential (AEP) [216] and the visual evoked potential (VEP) [217], respectively. The notion that these non-invasive methods induce LTP-like cortical plasticity is supported by enhanced fMRI activation and increased levels of glutamatergic metabolites in visual cortices following high frequency visual stimulation [218]. Modulation of motor cortex excitability has been demonstrated
through application of tDCS [219]. Furthermore, high-frequency repetitive TMS stimulation of the hand motor cortex can lead to lasting increases of motor-evoked potential (MEP) amplitudes [220]. Associative LTP-like plasticity is often modeled using the paired associative stimulation (PAS) paradigm [221, 222]. Here, potentiation of MEPs is induced when low-frequency TMS of the motor cortex is paired with electrical stimulation of the median nerve, thereby stimulating the same cortical region via two independent afferent pathways [223]. Sleep slow wave activity (SWA) is another potential EEG-based index of cortical synaptic plasticity [224-228]. Recent studies indicate that TMS and tDCS can alter SWA [229, 230] and that acoustic stimulation might enhance SWA during sleep [226].

Each of these methodologies can induce sustained change in cortical responsiveness in humans *in vivo*. While more research is needed to clarify the precise neural processes underlying these cortical plasticity indices and their fundamental properties such as test-retest reliability, they do exhibit core features of conventional LTP [220, 223, 231-238].

1.4.2 Plasticity of the visual evoked potential is a likely *in vivo* correlate of LTP-like cortical plasticity

The visual cortex can be activated non-invasively using visual stimulation, shows robust experience-dependent plasticity, and is a particularly attractive system for LTP-like cortical plasticity examinations [239, 240]. The VEP is a stimulus-synchronized averaged EEG-signal that mainly reflects postsynaptic potentials in the visual cortices [241-243]. The VEP is widely used in the clinic to measure the functional integrity of the visual pathway, and is, when elicited by a reversing checkerboard pattern, characterized by three major peaks, as shown in Figure 2 on page 27: the C1, the P1, and the N1 [244]. These peaks are usually present irrespective of attention and task, and their amplitudes and latencies vary by the physical properties of the visual stimuli [245]. The neural sources of pattern-reversal VEPs are not fully clarified, however, the C1 may mainly reflect postsynaptic activity in the primary visual cortex (V1), while the P1 and N1 likely mirror postsynaptic potentials in both striate and extrastriate cortices [244, 246].
Studies in rodents show that repeated visual stimulation can cause lasting increases or plasticity of VEP amplitudes [231-233]. Further detailed experiments have demonstrated that the VEP plasticity is persistent, stimulus-specific, and depends on NMDA and AMPA receptors, and on protein kinase ζ [231-233], i.e., core features of canonical LTP. Moreover, plasticity of the VEP induced by repetitive visual stimulation and LTP induced by repetitive electrical thalamo-cortical stimulation in rodents mimic and mutually occlude one another, indicating that mechanisms of LTP are necessary and sufficient to account for VEP plasticity [232]. VEP plasticity induced by repeated visual stimulation has also been demonstrated in several human studies, as illustrated by Figure 2 [19, 217, 247-250]. Although the precise neural substrate for human VEP plasticity remains to be fully clarified, the amplitude modulations show specificity for the frequency and pattern of the stimulation [251], and can be enhanced by the partial NMDA receptor agonist D-cycloserine [252]. Together, the rodent and human studies strongly suggest that plasticity of the VEP represents a valid and accessible non-invasive method for studying cortical synaptic function closely related to LTP.

1.4.3 Cortical thickness changes measured by magnetic resonance imaging

MRI is a non-invasive method that relies on varying magnetic properties of tissue types to generate images of biological tissue [253]. MRI is the instrument of choice in brain structure studies since it does not emit ionizing radiation, allows for imaging of the same individual multiple times, and is sensitive to subtle structural changes. Commonly used strategies for analyzing structural cortical changes are voxel-based morphometry (VBM), which examines differences in an exploratory manner in every voxel (box-shaped units which make up the MRI

Figure 2. The visual evoked potential (VEP), with C1, P1, and N1 amplitudes. The figure shows averaged VEP plots before (black line) and 2 to 28 minutes after (color-coded) 10 minutes of plasticity-inducing checkerboard reversal stimulation. Adapted from Normann C, et al, Biological Psychiatry 2007. Reprinted with permission from Elsevier.
image) across the brain, and region of interest (ROI) analyses, where average measures from a priori defined brain regions are calculated. Recently developed surface-based approaches allow for separate examinations of cortical surface area and cortical thickness, which are likely genetically and phenotypically independent measures [254]. Freesurfer is a popular and freely available software that automatically segments the cortical surface (Figure 3) [255]. The cortical thickness estimates of Freesurfer have been validated against histological analysis [256] and manual tracing [257, 258].

1.4.4 Is there a relationship between alterations in cortical plasticity and cortical thickness changes?

Thinning of the cerebral cortex is observed in healthy aging and in psychiatric disorders [257, 259-261]. Moreover, previous studies have linked greater cortical thinning to cognitive impairment [262], development of Alzheimer's disease [263], and conversion to psychosis in high-risk individuals [264]. Yet, there are currently no biomarkers that can predict future rate of cortical thinning. Furthermore, the neurobiological substrate underlying changes in cortical thickness remains to be clarified. However, altered synaptic function and plasticity and loss of dysfunctional synapses are leading candidate mechanism in age-related cognitive decline and psychiatric illnesses [145, 265-272].

VEP plasticity is a promising in vivo index for cortical synaptic processes, as discussed in Section 1.4.2 and impairments in VEP plasticity might indicate reduced capacity for cortical LTP-like plasticity, and, possibly, a shift towards LTD-like synaptic plasticity. Reduced LTP-like plasticity and increased LTD-like plasticity are associated with greater loss of synapses [146-148]. Synapses constitute approximately 10% of total cortical volume in the mammalian brain and loss of synapses with secondary retraction of dendrites and glial processes and decreased neuronal size could lead to cortical thinning detectable with MRI [259, 273, 274]. To
date, no study has examined whether non-invasive indices of synaptic function and plasticity can predict future changes in cortical thickness.
2. Aims of the thesis

The overall goal of the present thesis was to contribute to greater understanding of cortical structure and plasticity alterations in BD type II and their longitudinal trajectories. The main aims of the thesis were:

I. To longitudinally examine whether VEP plasticity impairment in BD type II is a stable disease trait or mood-state related and to assess the relationship between VEP plasticity and saliva cortisol.

II. To longitudinally investigate whether cortical thickness abnormalities in BD type II are stable disease traits and to examine the effects of mood episodes on cortical thickness changes.

III. To test the hypothesis that VEP plasticity can predict the rate of future cortical thinning in individuals with BD type II and in HCs.
3. Materials and methods

3.1 Setting

This doctoral work collected and analyzed longitudinal and cross-sectional data from examinations of cortical plasticity and structure in subjects with BD type II and healthy individuals at baseline and on average 2.4 years later, at follow-up. The examinations were conducted at the Department of Psychosomatic Medicine, Oslo University Hospital. Results from the examinations at baseline have been published previously [19, 20].

3.2 Study participants

The study participants were included and underwent baseline examinations between January 2009 and June 2010. Forty-three HCs were recruited through local advertising, and forty individuals with BD type II were recruited from psychiatric outpatient clinics in the Oslo area and from the Department of Psychosomatic Medicine, Oslo University Hospital.

The inclusion criterion for the patient group was a diagnosis of DSM-IV-TR-defined BD type II (Table 1 on page 14). HCs with previous or current psychiatric illness were excluded from the study. The exclusion criteria for all participants were:

- age below 18 or above 50 years
- previous head injury with loss of consciousness for more than one minute
- history of neurological or other severe chronic somatic disorder
- pregnancy
- contraindications for MRI examination.

Participants were invited back for follow-up examinations on average 2.4 years later. Reasons for not participating in the follow-up study were: moving out of the area (1 patient, 5 HCs), premalignant condition diagnosed during follow-up (1 HC), did not attend or withdrew consent (6 patients, 2 HCs), claustrophobia (2 patients) and pregnancy (1 HC). Additional patients and HCs were recruited at follow-up to increase sample size for cross-sectional analyses (Figure 4 on page 33).

Before study inclusion was initiated in 2009, sample size estimation for VEP plasticity examinations was performed using Altman’s nomogram [275]. Based on a study of VEP plasticity in subjects with MDD [251], it was estimated that 18 subjects with BD type II and 18
HCs would be needed to detect a group difference in VEP plasticity of 25%, with $\alpha=0.05$ and $\beta=0.20$. When the MRI study at baseline was planned, there were no published studies of cerebral cortical thickness or surface area in subjects with BD-II. Thus, no formal sample size estimation was performed for the MRI studies. However, since Lyoo et al. found significantly reduced cortical thickness in a mixed sample of 25 subjects with BD-I and BD-II relative to 21 HCs [276], the aim was to include at least 25 subjects with BD-II and 25 HCs. Since there were significant group differences at baseline, we expected to find group effects with similar sample sizes at follow-up. When this work was planned, there were no longitudinal studies of VEP plasticity or cortical thickness in BD type II and no formal sample size estimation were performed for the longitudinal parts of the present thesis.

Participants were invited to undergo both VEP plasticity examinations and MRI scans. However, the VEP plasticity and MRI samples of the present thesis are not identical, since not all participants completed both VEP and MRI examinations at baseline and follow-up, e.g., due to claustrophobia. Furthermore, not all participants underwent VEP plasticity examinations at baseline since patients were recruited in collaboration with another study at the Department of Psychosomatic Medicine, Oslo University Hospital. In this study, individuals with BD type II or borderline personality disorder underwent the same clinical and MRI protocols as in the present thesis; however, these participants did not initially undergo VEP plasticity examinations. Figure 4 on page 33 shows a flowchart for the participants of the VEP plasticity (A) and MRI (B) examinations of this thesis.

Paper I included 18 individuals with BD type II and 33 HCs who completed VEP plasticity examinations at baseline and follow-up. Two patients and four controls were excluded from the analyses owing to technical issues during EEG recording and insufficient data quality, thus the longitudinal sample comprised 29 controls and 16 patients. Moreover, 16 additional patients and one new control were included at follow-up to further assess the relationship between VEP plasticity and saliva cortisol and mood state. Owing to technical issues and insufficient data quality, three of the new patients and the new control were excluded from the analyses. The cross-sectional sample at follow-up thus consisted of 29 individuals with BD type II and 29 HCs.

Paper II included 29 patients and 33 healthy individuals who completed MRI examinations at baseline and follow-up (longitudinal sample). Two of the longitudinal patients were not included in the previously published baseline analyses, since one patient was examined after the cross-sectional baseline analyses were finalized, and the other patient was excluded from
the baseline analyses due to alcohol abuse, as requested by one the reviewers of the cortical thickness study of baseline [20]. There were seven new patients and two additional HCs included at follow-up, resulting in a cross-sectional sample at follow-up of 36 patients and 35 HCs.

Figure 4. Flowchart of participants included in this thesis. (A) Participants included in VEP plasticity analyses. (B) Participants included in cortical thickness analyses. The samples in A and B are overlapping.
Paper III included 15 patients and 32 healthy individuals who completed VEP plasticity examinations at baseline and MRI examinations both at baseline and follow-up. Demographic and clinical characteristics of the samples in each study are summarized in Table 2.

<table>
<thead>
<tr>
<th></th>
<th>Paper I</th>
<th>Paper II</th>
<th>Paper III</th>
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<tbody>
<tr>
<td></td>
<td>BD type II</td>
<td>HCs</td>
<td>BD type II</td>
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<tr>
<td>n</td>
<td>Long*</td>
<td>Cross</td>
<td>Long*</td>
</tr>
<tr>
<td>Age</td>
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<td>35.6 (9.6)</td>
<td>32.7 (7.5)</td>
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<tr>
<td>Females</td>
<td>9 (56)</td>
<td>19 (66)</td>
<td>16 (55)</td>
</tr>
<tr>
<td>MADRS</td>
<td>11.9 (6.5)</td>
<td>8.8 (6.8)</td>
<td>1.3 (2.2)</td>
</tr>
<tr>
<td>YMRS</td>
<td>3.0 (3.4)</td>
<td>2.4 (2.5)</td>
<td>0.3 (0.8)</td>
</tr>
<tr>
<td>Illness duration</td>
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<td>18.0 (7.3)</td>
<td>n.a.</td>
</tr>
<tr>
<td>Social phobia</td>
<td>4 (25)</td>
<td>10 (34)</td>
<td>n.a.</td>
</tr>
<tr>
<td>Panic disorder</td>
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<td>18 (62)</td>
<td>n.a.</td>
</tr>
<tr>
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<td>2 (7)</td>
<td>n.a.</td>
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<tr>
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<tr>
<td>Unmedicated</td>
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<td>7 (24)</td>
<td>n.a.</td>
</tr>
<tr>
<td>Mood stabilizers</td>
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<td>16 (55)</td>
<td>n.a.</td>
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<td>n.a.</td>
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<tr>
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<td>1 (3)</td>
<td>n.a.</td>
</tr>
<tr>
<td>Antipsychotics</td>
<td>1 (6)</td>
<td>3 (10)</td>
<td>n.a.</td>
</tr>
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Table 2. Mean (SD) is shown for continuous variables, and n (%) is shown for categorical variables. Long: Longitudinal sample. Cross: Cross-sectional sample at follow-up. *Characteristics at baseline.

### 3.3 Clinical assessments

All participants were examined by senior psychiatrists at the Department of Psychosomatic Medicine, Oslo University Hospital, which specializes in evaluation and treatment of mood disorders. All patients and healthy subjects underwent a thorough clinical examination that included general biochemistry screening, psychometric measures, saliva cortisol measures, and neuropsychological testing. In addition, all MRI scans were examined by a neuroradiologist to rule out structural intracranial pathology. The following clinical measures were used in the studies of the thesis:
The Mini-International Neuropsychiatric Interview (M.I.N.I.)
The M.I.N.I., DSM-IV criteria version 5.0.0 [277], was used to determine axis I diagnoses and psychiatric comorbidities. The M.I.N.I. is a structured diagnostic interview designed for clinical and research settings and several studies have shown that the inter-rater correlation, sensitivity, and specificity of the M.I.N.I. are good to excellent [277]. The psychiatrists that performed the clinical examinations for the present study had attended training courses in use of the M.I.N.I.

The Stanley Foundation Bipolar Network Entry Questionnaire (NEQ)
The NEQ, formerly known as the Patient Questionnaire and the Clinician Questionnaire [278], was applied as a semi-structured interview to obtain demographic and supplementary information, including duration of illness, lifetime number of major depressive and hypomanic episodes, medication, education level, tobacco use, and body mass index.

Montgomery–Asberg Depression Rating Scale (MADRS)
MADRS [279] is a 10-item checklist that is widely used to measure depression severity. Each item is scored on a scale from 0-6, thus resulting in a total score between 0-60. Inter-rater correlation for total MADRS score are between 0.76 and 0.97 [279, 280]. All raters in the present study had been trained in the use of MADRS and had an intraclass correlation coefficient (ICC)\textsubscript{1,1} \geq 0.80.

Young Mania Rating Scale (YMRS)
YMRS is a checklist of 11 items designed to measure the severity of manic symptoms [281]. The items are scored from 0-4 or 0-8 and the total YMRS score ranges from 0-60. In the original work of Young et al., there was an inter-rater correlation of 0.93 for total YMRS [281]. All raters in the present study had been trained in the use of YMRS, and there was strong agreement that no patient had a previous or current episode of mania.

The Alcohol Use Scale (AUS) and Drug Use Scale (DUS)
The AUS and DUS each consist of one item where the clinician rates the person's substance use from 1-5: 1 = abstinent, 2 = use without impairment, 3 = abuse, 4 = dependence, and 5 = dependence with institutionalization [282, 283]. The AUS and DUS have high reliability and validity [283]. The ICC was not tested specifically for this study.
3.4 VEP plasticity

Experimental paradigm
The experimental paradigm described by Normann et al. [251] was used at baseline [19] and follow-up. VEPs were evoked by checkerboard reversals (check size=0.5°; 2 reversals/second) in two premodulation blocks before and six blocks after a plasticity-inducing modulation block. In each pre- and postmodulation block, 40 checkerboard reversals were presented within 20 seconds. In the modulation block, VEPs were evoked by checkerboard reversals (check size=0.5°; 2 reversals/second) for 10 minutes. A gray screen was displayed between checkerboard stimulations. The visual stimuli were presented with E-Prime 1.1 (Psychology Software Tools, Sharpsburg, Pennsylvania) on a 24-inch LCD screen.

Recording and analysis of the VEP
VEP plasticity was assessed using EEG data from the Oz electrode. The baseline examination included MMN and oddball paradigms, and EEG activity was therefore recorded from 15 monopolar silver/silver chloride electrodes for analyses of these paradigms. However, the follow-up examination only comprised the VEP plasticity paradigm and only the O1, Oz, and O2 electrodes at the occipital head were therefore used. All impedances were maintained below 5 kΩ and the ground and reference electrodes were attached to the forehead (AFz). Eye movements were recorded with bipolar electrodes placed at the sub- and supraorbital regions and at the lateral canthi of each eye. EEG activity was recorded at 250 Hz with an amplifier band-pass of 0.05–100 Hz. Offline EEG analysis was conducted using EEGLAB43 [284], run on MATLAB 7.6.0. (MathWorks, Natick, Massachusetts). The EEG was first high-pass filtered at 1 Hz and segmented into epochs starting 150 milliseconds before and continuing 350 milliseconds after the onset of each checkerboard reversal. All epochs containing eye movement-related activity were removed from analyses. Epochs were then shortened (−50 to 350 milliseconds) and baseline-corrected (−50 to 0 milliseconds), and epochs with amplitudes exceeding ± 100 µV on any of the occipital channels (O1, Oz, O2) were rejected. The epoched EEG was finally low-pass filtered at 30 Hz and averaged to block-specific VEPs. Peak amplitudes and latencies for the C1, P1, N1, and the P1–N1 peak-to-peak amplitudes were obtained from the Oz electrode; amplitudes were measured relative to the 50 milliseconds baseline.
3.5 Saliva collection and cortisol analysis

Saliva samples for cortisol analysis were collected using Salivette® Cortisol swabs (Sarstedt AG & Co, Nümbrecht, Germany) and analyzed with a Cortisol Saliva Luminescence Immunoassay (IBL International, Hamburg, Germany) according to the manufacturers’ instructions. Saliva samples were obtained the day after the VEP experiment at three times: immediately after awakening in the morning, 30 minutes after the first collection, and at 12:30 PM. Participants were instructed to not brush teeth and to refrain from physical activity, nicotine, and caffeine before saliva collection and to not eat or drink the last 30 minutes before the samples were obtained. Saliva cortisol was averaged across the three collections. Cortisol awakening response was defined as saliva cortisol 30 minutes post-awakening minus cortisol at awakening.

3.6 MRI acquisition and analysis

Imaging at baseline and follow-up was performed on the same 3T Philips Achieva Scanner (Philips Healthcare, Eindhoven, the Netherlands) using an eight-channel SENSE head coil. The pulse sequence used for cortical thickness analyses was a T1-weighted three-dimensional turbo field echo (TFE) sequence (repetition time (TR)/echo time (TE)=8.4 millisecond /2.3 milliseconds, field of view (FOV)=256mm × 256mm × 220mm, 1mm isotropic resolution, TA=7 minutes 40 seconds). The MRI protocol also included a DTI sequence and sequences for hippocampal and neocortical MR spectroscopy. Total scanning time was approximately 70 minutes.

Measurement and analyses of cortical thickness

All data sets were processed blindly and analyzed using Freesurfer software version 5.3, which automatically reconstructs cortical surfaces from T1-weighted MRI-images [285-291]. Briefly, processing steps include removal of non-brain tissue [292], automated Talairach transformation, and intensity correction. Information about intensity and continuity from the three-dimensional volume is used in segmentation and deformation procedures to reconstruct a gray/white matter boundary across the brain [285]. Cortical surfaces are then inflated, registered to a spherical atlas, and gyral and sulcal regions are identified automatically [292]. Reconstructed data sets were visually inspected for accuracy at several points along the processing pipeline, and segmentation errors were manually corrected, re-processed, and re-inspected. Cortical thickness maps were obtained for each participant by calculating the distance between the gray and white matter surfaces at each vertex.
**Longitudinal analyses**

For longitudinal analyses, the longitudinal stream implemented in Freesurfer was used to prepare surfaces for comparisons of baseline and follow-up examinations [293]. This procedure processes longitudinal data with common information from a template created for each subject, thereby reducing measurement noise and increasing precision. Temporal data within each subject was then reduced to rate of change maps (mm/year= thick2-thick1/time2-time1) using the `long_mris_slopes` function in Freesurfer’s longitudinal two-stage model.

Before statistical analysis, cortical thickness maps processed for cross-sectional and longitudinal analyses were smoothed with a full width of half maximum Gaussian kernel of 20mm, as in the previously published baseline analyses [20], to improve signal-to-noise ratio and statistical power [294, 295].

### 3.7 Statistical analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) for Windows, version 24.0, and a two-tailed $p$ value of <.05 was considered significant. To test for differences in demographic and clinical variables between patients with BD type II and HCs, independent samples $t$-tests were performed for continuous variables, and the $\chi^2$ test and the Fisher’s exact test were performed for categorical variables.

Main analyses relied on the general linear model (GLM), and analyses of variance and covariance (ANOVAs and ANCOVAs) were used for group comparisons and associations with clinical variables, after testing the assumption of homogeneity of regression slopes.

In **Paper I** repeated-measure ANOVAs were conducted in order to test the effect of the modulation block on VEP latencies and amplitudes. Bonferroni correction was employed when testing the plasticity effect on each of the postmodulation blocks. Bivariate correlation (Pearson's $r$) was used to test relationships between VEP plasticity scores and clinical variables (mood state and saliva cortisol). The temporal stability of VEP plasticity was examined using ICC$_{3,1}$.

In **Paper II** mean MRI-based estimated cortical thickness (in mm) and mean rates of thickness change (in mm/year) were calculated for each participant in clusters showing significant between-group differences, and these values were used to calculate effect sizes. GLMs, with Bonferroni-corrected post-hoc tests, were then employed to test the effect of mood episodes on cluster mean values in the patient group, while controlling for age and sex. In order to test for
possible outlier effects, analyses were reran after removing subjects with studentized residuals of $>|2.0|$.

In **Papers II** and **III**, whole-brain surface-based analyses were performed using vertex-wise GLMs (*mri_glmfit* option), as implemented in the longitudinal two-stage model in Freesurfer. Main effects of group were tested by contrasting the patients and HCs while controlling for age and sex. In **Paper II**, further surface-based analyses were conducted pairwise contrasting HCs and patients with few mood episodes, HCs and patients with many mood episodes, and patients with few and patients with many mood episodes. To reduce the probability of type I errors, surface-based analyses were corrected for multiple comparisons using cluster size inference by means of Z Monte Carlo simulations, as implemented in Freesurfer [296, 297]. The initial cluster-forming threshold employed was $p<.05$. In **Paper III**, effects of VEP plasticity on cortical thickness changes were tested by adding the EEG-based plasticity value (premodulation VEP amplitude subtracted from postmodulation amplitude) as a continuous variable in the GLMs. Statistical significance was assessed through permutation-based non-parametric tests (Permutation Analyses of Linear Models - PALM) [298]. Threshold-free cluster enhancement (TCFE), which incorporates spatial neighborhood information without the use of a predefined threshold, was performed and familywise error rate (FEW)-corrected $p$ value images were produced after 5000 permutations.

### 3.8 Ethical considerations

The Regional Ethical Committee of South-Eastern Norway approved the study, and all subjects provided written informed consent to participate after receiving a complete description of the study given orally and in writing. The applied methods were not associated with risk of harm or significant discomfort. All participants were informed about their right to withdraw from the study at any time, without consequences for future treatment.
4. Summary of papers

4.1 Paper I: Longitudinal and cross-sectional investigations of long-term potentiation-like cortical plasticity in bipolar disorder type II and healthy individuals

**Background:** Visual evoked potential (VEP) plasticity is a promising assay for non-invasive examination of long-term potentiation (LTP)-like synaptic processes in the cerebral cortex. Our research group previously found reduced VEP plasticity in BD type II compared to healthy controls (HCs) at baseline [19]. Here, we conducted longitudinal and cross-sectional investigations of VEP plasticity in individuals with BD type II and HCs with the following main aims: 1) to test the reproducibility of impaired VEP plasticity in BD type II and to assess the relationship between mood state and plasticity, 2) to examine the relationship between saliva cortisol and VEP plasticity in BD type II and HCs, and 3) to examine the temporal stability of VEP plasticity.

**Methods:** VEP plasticity was assessed at baseline and 2.2 years later, at follow-up. The longitudinal sample with VEP data from both time points comprised 29 controls and 16 patients. VEP data was available from 13 additional patients at follow-up (total \(n=58\)). VEPs were evoked by checkerboard reversals in two premodulation blocks before and six blocks after a plasticity-inducing block of prolonged (10 minutes) visual stimulation. VEP plasticity was computed by subtracting premodulation VEP amplitudes from postmodulation amplitudes. Saliva samples for cortisol analysis were collected immediately after awakening in the morning, 30 minutes later, and at 12:30 PM, at follow-up.

**Results:** We found reduced VEP plasticity in BD type II, that impaired plasticity was present in the euthymic phases of the illness, and that VEP plasticity correlated negatively with depression severity. There was a positive association between VEP plasticity and saliva cortisol in HCs, possibly reflecting an inverted U-shaped relationship between cortisol and synaptic plasticity. VEP plasticity exhibited moderate temporal stability over a period of 2.2 years.

**Conclusion:** The results provide additional evidence for impaired LTP-like cortical plasticity in BD type II. VEP plasticity is an accessible method, which may help elucidate the pathophysiological and clinical significance of synaptic dysfunction in psychiatric disorders.
4.2 Paper II: Mood episodes are associated with increased cortical thinning: a longitudinal study of bipolar disorder type II

Background: Previous studies found evidence for thinner frontotemporal cortices in bipolar disorder (BD), yet whether this represents a stable disease trait or an effect of mood episodes remains unknown. Here we assessed the reproducibility of thinner frontotemporal cortices in BD type II, compared longitudinal changes in cortical thickness between individuals with BD type II and healthy controls (HCs), and examined the effect of mood episodes on cortical thickness change.

Methods: Thirty-three HCs and 29 individuals with BD type II underwent MRI at baseline, and 2.4 years later, at follow-up. Cross-sectional and longitudinal analyses of cortical thickness were performed using Freesurfer, and relationships with mood episodes from baseline to follow-up were assessed.

Results: Individuals with BD type II had thinner left and right prefrontal and left temporal cortex clusters at follow-up consistent with baseline results [20]. Both groups showed widespread longitudinal cortical thinning, and patients had increased thinning in a left temporal cortex cluster compared to HCs. Patients with more (>2) depressive episodes between baseline and follow-up had greater left temporal cortical thinning than patients with fewer depressive episodes. In addition, patients with more depressive episodes had greater thinning in bilateral ventromedial prefrontal clusters relative to HCs, yet these results did not survive correction for multiple comparisons.

Conclusion: Together, these findings support reduced frontotemporal cortical thickness in BD type II and provide preliminary evidence for an association between depressive episodes and increased cortical thinning.
4.3 Paper III: Cortical plasticity is associated with cortical thinning in healthy individuals and bipolar disorder: a longitudinal study.

**Background:** The precise mechanisms underlying cerebral cortical thinning in aging and psychiatric illnesses remain to be clarified, yet aging- and synaptic dysfunction-related loss of synapses are potential substrates. We used long-term potentiation (LTP)-like plasticity of the visual evoked potential (VEP) as an index of cortical synaptic function and hypothesized that plasticity at baseline would predict future cortical thinning in healthy individuals and subjects with bipolar disorder (BD) type II.

**Methods:** Thirty-two healthy individuals and 15 individuals with BD type II underwent electroencephalography-based measurement of VEP plasticity and 3T magnetic resonance imaging at baseline and 2.3 years later, at follow-up. The relationships between VEP plasticity at baseline and changes in cortical thickness from baseline to follow-up were examined using Freesurfer and the Permutation Analysis of Linear Models tool.

**Results:** The analyses showed a negative association between plasticity of the N1 VEP amplitude at baseline and rate of thinning in widespread cortical regions in the whole sample ($n=47$) and in healthy individuals, indicating greater thinning over time in subjects with less N1 plasticity ($p_{FWER}<.05$). There was a positive association between P1 plasticity at baseline and cortical thinning in the whole sample and in patients ($p_{FWER}<.05$); these findings were not significant after removing outliers from the analysis.

**Conclusion:** These results indicate that VEP plasticity is associated with future rate of cortical thinning in healthy individuals and in BD type II, supporting the hypothesis that synaptic dysfunction is related to cortical thinning. Further longitudinal studies are needed to confirm these findings.
5. Discussion

5.1 Discussion of main findings

5.1.1 Impaired VEP plasticity in bipolar disorder type II

The present study of VEP plasticity in individuals with BD type II and HCs had three main findings. First, we reproduced impaired VEP plasticity in BD type II at the follow-up examinations. We also found that VEP plasticity was reduced in euthymic patients and was negatively correlated with depression severity. Second, we showed that saliva cortisol was increased in BD type II, that VEP plasticity remained impaired in patients after controlling for saliva cortisol, and that saliva cortisol was positively correlated with plasticity of the VEP in HCs. Finally, plasticity of the VEP exhibited moderate temporal stability when the examinations at baseline and follow-up were compared. These findings support a growing body of evidence indicating that mood disorders are associated with impaired synaptic function and plasticity [11, 15, 114, 115].

Here, the results and how they contribute to greater understanding of LTP-like cortical plasticity impairments in BD type II will be discussed by addressing the following questions:

1. What mechanisms might underlie LTP-like plasticity impairments in BD type II?
2. Does impaired LTP-like cortical plasticity represent a state or trait phenomenon in BD type II?
3. Can VEP plasticity inform us about plasticity abnormalities in cortical regions implicated in BDs?

What mechanisms might underlie LTP-like cortical plasticity impairments in BD type II?

The precise mechanisms underlying LTP-like cortical plasticity impairments in BD type II remain to be elucidated. However, these impairments may arise from interactions between environmental risk factors, such as stress [299] and genetic variants associated with BDs [153]. Stressful life events and chronic psychological stress are strong predictors of outcome in the BDs [300, 301], and individuals with BDs show accelerated telomere shortening [94, 302], indicating increased cumulative stress exposure [303]. In Paper I we found that patients with BD type II had increased levels of saliva cortisol relative to HCs, which might reflect increased stress levels. This finding is in line with two recent meta-analyses of cortisol levels in BDs [304, 305]. Glucocorticoids have well-established effects on synaptic function and plasticity, which
oftentimes follow an inverted U-shaped curve: at low and high levels, glucocorticoids can impair synaptic function and plasticity, whereas normal glucocorticoid concentrations facilitate synaptic plasticity processes, such as LTP [306, 307]. It is therefore possible that elevated cortisol levels could contribute to VEP plasticity reductions in individuals with BD type II. However, we did not find any significant associations between saliva cortisol and VEP plasticity in patients and plasticity impairments remained significant after covarying the analyses for cortisol levels. Although we cannot rule out that measurement of cortisol levels over longer periods than one day would have revealed significant associations between cortisol and VEP plasticity in patients, the results indicate that other mechanisms than cortisol elevation might underlie the VEP plasticity impairments in BD type II.

In HCs, on the other hand, there were significant positive correlations between VEP plasticity and averaged saliva cortisol and the cortisol awakening response. Although speculative, these positive associations could reflect the ascending part of the inverted U-shaped relationship between cortisol and LTP-like plasticity. This hypothesis could be tested further in future studies of VEP plasticity by including more individuals with higher stress and cortisol levels than the present work.

Furthermore, ample evidence demonstrates that inflammatory mediators influence synaptic transmission and plasticity [308], and that pro-inflammatory cytokines are involved in induction and maintenance of hippocampal LTP [309, 310]. The low-grade pro-inflammatory state found in patients with BDs, even during euthymia, might be associated with VEP plasticity impairments, and increasing levels of cytokines during mood episodes could contribute to further plasticity deteriorations in depressed patients [311-315]. Yet whether and how immune processes affect VEP plasticity in BD type II remains to be investigated in future studies.

Changes in BDNF levels, which is an important regulator of synaptic transmission and LTP, might also contribute to impairments in LTP-like cortical plasticity [316, 317]. Results from a meta-analysis indicate that patients with BD type I and II have BDNF levels comparable to HCs during euthymia, lower BDNF levels during manic and depressed states, and the lowest levels when episodes are severe (although it was not possible to separate subtypes of BDs for these latter analyses) [318]. In patients with MDD, reduced BDNF levels are found during depressive episodes [319, 320], while levels may normalize after remission [319]. One study reported impairments in PAS-induced plasticity of MEPs in severely depressed patients with MDD, but found no significant correlation between plasticity and BDNF levels, which might suggest other underlying mechanisms for impaired plasticity, at least in MDD [321]. BDNF levels were not
measured in the present study and it remains to be clarified whether decreased BDNF levels during depression contributes to VEP plasticity impairment in BD type II.

BDs-associated genetic variants might also contribute to VEP plasticity impairments in BD type II, as calcium signaling and glutamatergic synaptic function are prominent among the genetic risk pathways [322-327]. BDs risk was associated with loci in genes coding for NMDA receptor subunits (GRIN2A) and other synaptic components (RIMS1, ANK3) in the largest GWAS to date [328]. Furthermore, findings from this and previous GWASs implicate dysregulated calcium-signaling in BDs since disease risk is associated with genetic variants within CACNA1C and CACNB2 (which encode subunits of postsynaptic voltage-gate calcium channels (VGCC)) and CREB (which encodes a transcription factor important for translating activation of VGCCs into lasting changes in synaptic function) [90, 327-330]. Whether and how BDs genetic risk variants affect plasticity of the VEP remains to be clarified.

Moreover, epigenetic mechanisms, which are heritable, but reversible modulations of gene expression in response to environmental changes, might be implicated in BDs pathophysiology [331, 332]. For instance, one study found reduced expression of the BDNF gene, and hypermethylation of the BDNF promoter in BD type II [27], while another study reported downregulation of GABA-ergic and BDNF genes in postmortem cortical tissues from individuals with BD [333]. How modifications in the neural epigenome might affect VEP plasticity in BDs remains unknown. However, emerging evidence indicates that mediation of gene-environmental interactions through reprogramming of gene expression is required for activity-dependent regulation of neuronal differentiation, maturation, and plasticity [334].

**Does impaired LTP-like plasticity represent a state or trait phenomenon in BD type II?**

The clarification of whether putative synaptic plasticity abnormalities represent state or trait-markers in BD type II may increase our understanding of mechanisms associated with vulnerability to and recovery from acute mood episodes, and may suggest treatment targets for preventing or treating acute mood episodes, and markers of treatment response.

Indirect measures of synaptic plasticity, such as neurocognitive function and NMDA receptor-dependent memory, are often impaired in BDs [335-337]. Impairments are commonly present in euthymia, with evident worsening during mood episodes [338-340]. These observations suggest that LTP-like plasticity impairments might represent both state and trait features in BDs and are consistent with the results of Paper I.
Few studies have examined the association between LTP-like plasticity impairments and disease episodes in mood disorders. Impaired VEP plasticity has been demonstrated in severely depressed subjects with MDD, however, it is not known whether the impairment normalizes after remission [251]. One study found impairments in PAS-induced plasticity in depressed individuals with MDD, while remission resulted in normalization of plasticity measures [341]. Furthermore, plasticity deficits persisted in non-remitted patients at follow-up examinations [341]. These results suggest that LTP-like plasticity impairments might be state dependent, at least in MDD. In contrast, the results from Paper I show reduced VEP plasticity in patients with BD type II relative to HCs at baseline and at follow-up during euthymia. These findings suggest that impaired VEP plasticity could be a stable trait in adults with BD type II. However, we also found that VEP plasticity correlated negatively with MADRS score, indicating less plasticity in more severely depressed patients. This finding suggests depression-related effects on VEP plasticity in the patients. There were no significant associations between hypomania severity and VEP plasticity, yet the hypomanic symptoms of the patients included in this thesis were generally mild. This negative finding should therefore be considered cautiously and future studies are needed to clarify the relationship between hypomania and VEP plasticity.

Altogether, the results of Paper I support both trait- and state-related impairments of LTP-like cortical plasticity in BD type II. However, it remains to be determined whether impaired plasticity is present in individuals with genetically increased risk for BD and precedes illness onset. Moreover, it is not possible to conclude on the temporal relation between depression severity and LTP-like plasticity impairments based on the correlation reported in Paper I. Thus, whether depression-related effects impair LTP-like cortical plasticity, or whether reduced plasticity causes depressive episodes remain to be clarified.

**Can VEP plasticity inform us about plasticity abnormalities in cortical regions implicated in BD?**

The results of the present thesis and previous studies indicate that VEP plasticity is impaired in BD type II and MDD [19, 251]. However, based on the current understanding of mood regulation in humans [342, 343], it is unlikely that impaired visual cortex plasticity is a central pathophysiological mechanism in mood disorders. An important question is therefore whether VEP plasticity reflects plasticity in brain regions believed to be important in mood disorders, such as prefrontal and temporal cortices. No previous study has examined the association between VEP plasticity and plasticity in prefrontal and temporal regions implicated in BD pathophysiology. However, one study found that VEP plasticity correlated significantly with
TMS-induced LTP-like plasticity in the motor cortex [344]. Moreover, experimental data indicate that synaptic mechanisms underlying LTP might be shared between visual and prefrontal cortices [345]. Furthermore, the significant negative correlation between VEP plasticity and depression severity reported in Paper I suggests that VEP plasticity might be correlated with plasticity in regions important for mood regulation. Together, these findings indicate that measurements of LTP-like plasticity in the visual cortex might, at least to a certain extent, reflect synaptic function and plasticity in other cortical regions. Further research could assess the relationship between VEP plasticity and PAS-induced LTP-like plasticity in the dIPFC [346] and between VEP plasticity and LTP-like plasticity in the auditory cortex induced by auditory tetanus [216].

Another outstanding question is whether BD is associated with synaptic abnormalities mainly in cortical regions implicated in mood regulation, e.g., in frontal and temporal cortices, or also in other cortices, such as the visual cortex. Post mortem studies in BD have found evidence for synaptic impairments in several cortical regions, including prefrontal, temporal, and visual cortices [155, 156, 158, 160, 347, 348], indicating that cortical synaptic alterations in BD are widespread and not confined to regions believed to be important for mood regulation. Examinations of LTP-like plasticity in the visual cortex could therefore be informative for understanding plasticity abnormalities in other cortical regions. Nevertheless, there is clearly a need for more research to increase our understanding of the relationship between VEP plasticity and LTP-like plasticity in cortices implicated in mood disorders.

5.1.2 Increased cortical thinning in bipolar disorder type II

Current neural models of BDs highlight dysfunction in emotion processing and mood-regulation circuits [20, 21, 261, 276, 349]. Bilateral medial prefrontal and rostral anterior cingulate cortices, and their connections to medial temporal structures, such as amygdala and hippocampus, are implicated in automatic emotion processing, while dorsal regions of the PFC have consistently been linked to voluntary emotion regulation [343]. Functional and structural MRI studies of BDs demonstrate abnormalities in these neural circuits, which might account for emotional lability and dysregulation characteristic for many patients [7]. Our findings of reduced thickness in bilateral dorsolateral, dorsomedial and ventromedial prefrontal cortices, right perigenual anterior cingulate cortex, and left anterior temporal cortex are consistent with previous studies [7, 9, 343, 350, 351], and might represent a structural correlate for mood dysregulation.
Results from Paper II indicated increased rate of cortical thinning in patients with BD type II compared to HCs, suggesting that cortical changes may be progressive. Patients who experienced many depressive episodes between baseline and follow-up examinations had higher thinning rates compared to patients with few depressive episodes and to HCs. These findings suggest that increased cortical thinning might be an effect of mood episodes and, consequently, that further cortical thinning in BD type II might be preventable.

Here, the results and how they contribute to greater understanding of structural cortical changes in BD type II will be discussed by addressing the following questions:

1. Can mood episodes affect brain structure?
2. Does frontotemporal cortical thinning in BD type II represent a vulnerability marker or an illness effect?

**Can mood episodes affect brain structure?**

The longitudinal analyses in Paper II showed increased rate of cortical thinning in a left temporal region in patients with BD type II compared to HCs. Further analyses showed that thinning rate in this region was increased only in patients who experienced many (three or more) depressive episodes between baseline and follow-up, while patients who had less than three depressive episodes between baseline and follow-up scans did not show increased thinning rate compared to HCs. Although not statistically significant, patients who experienced many depressive episodes also experienced more hypomanic episodes during follow-up, which might suggest that mood episodes regardless of polarity could be associated with increased cortical thinning. Furthermore, uncorrected results indicated that patients who experienced many depressive episodes had increased temporal cortical thinning also in the right hemisphere, and in bilateral ventromedial prefrontal regions, yet these effects were not significant after correction for multiple comparisons.

Cross-sectional studies found that multi-episode patients had larger lateral ventricles compared to first-episode patients [352], and that number of mood episodes was negatively associated with hippocampal [353] and corpus callosum volume [354] and with gray matter volume (GMV) and cortical thickness in frontal and temporal areas [101, 355, 356]. Furthermore, a recent study examined whether a machine learning algorithm could distinguish individuals with BD from HCs based on gray and white matter density data, and found that patients who were identified with high certainty had a higher number of lifetime episodes of mania, indicating that structural abnormalities in BD might be associated with high illness burden [357]. However, the cross-
sectional design of these previous studies precludes conclusions about the direction of effects. Longitudinal imaging studies in BDs are few, yet available studies found that frontal volume decrease was only seen in patients who had at least one manic episode during follow-up compared to patients who did not have them [358]. In addition, longitudinal hippocampal and cerebellar gray matter loss was associated with number of manic/hypomanic and depressive episodes [359]. Another study from our research group found that number of depressive episodes during follow-up was associated with longitudinal volume decrease in the left dentate gyrus-CA4, and that bilateral hippocampal subfield volumes were negatively associated with markers of oxidative lipid damage [93]. Furthermore, a recent study of healthy individuals found that depressive symptoms at baseline were associated with increased cortical thinning in left frontal and bilateral superior temporal and supramarginal regions four years later [360].

These previous reports are in line with the results of Paper II and indicate that mood episodes can affect brain structure. However, two possible explanations might underlie the reported associations. Observable brain changes in BD type II might result from cumulative effects of multiple mood episodes. Alternatively, patients who experience many depressive episodes might belong to a subgroup with a more severe illness course from the onset, showing progressive neurobiological alterations, which might convey vulnerability for development of further episodes [361]. Although how mood episodes affect brain structure remains to be fully clarified, a recent longitudinal study found a significant association between cortisol levels and depression-related cortical thinning, suggesting a relationship between depression, stress, and cortical thinning [360]. Furthermore, increased levels of pro-inflammatory cytokines in BDs, and reduced levels of protective neurotrophic factors in later disease stages might together contribute to compromised cortical function and subsequent structural changes [311, 362, 363].

A recent study found that the serum of late-stage patients with BD type I induced a decrease in neurite density and cell viability in neuronal cultures [364]. This finding supports the systemic toxicity hypothesis, stating that the serum of BDs patients during mood episodes contain chemicals that could be toxic to neuronal structures [365]. Whether pro-inflammatory molecules can cross the blood-brain barrier (BBB) is unclear, yet it has been proposed that the BBB integrity is disrupted in BDs [366]. This could reduce the brain's protection from pro-inflammatory substances, which, together with decreased neurotrophic support, might compromise the integrity of neuronal circuits, and possibly result in increased cortical thinning in BDs [366].
Does frontotemporal cortical thinning in BD type II represent a vulnerability marker or an illness effect?

In Paper II we found that individuals with BD type II showed thinner bilateral frontal and anterior temporal cortices compared to HCs at follow-up, consistent with the baseline examination [20]. Furthermore, the neuroanatomical distribution of these findings mirrored results from the ENIGMA Bipolar Disorder consortium, which published the largest study of cortical thickness in BDs to date [21]. However, there was no difference in longitudinal cortical thinning in these clusters between individuals with BD type II and HCs, which could indicate that reduced frontal and anterior temporal cortical thickness might be a stable vulnerability marker for BD type II. Alternatively, increased cortical thinning in these regions might take place early in the disease course, and then reach a floor effect, resulting in no further measurable cortical thinning. In support of abnormal cortical development in BD, a study showed that youth with BDs and control youth exhibited different maturation trajectories of fronto-temporal-striatal neurocircuitry, and that BDs were associated with significantly greater thinning with age in the left frontal pole, compared to HCs [367]. Furthermore, another recently published study used automated analysis of cortical thickness to identify two subgroups of pediatric BDs patients early in the disease course [368]. One subgroup had increased thickness in widespread cortical regions, including frontotemporal regions, compared to control youth, while the other subgroup had thinner cortices, mainly in superior temporal and superior parietal regions [368]. Interestingly, greater cortical thickness was associated with increased response rate to antipsychotic treatment [368]. Studies of cortical thickness in non-affected family members of individuals with BDs suggest that the illness' genetic risk is associated with subtle thickness abnormalities in frontotemporal cortices [369-371]. Furthermore, a recent longitudinal study showed that offspring of BDs patients had increased frontal cortical thinning compared to HCs, although this finding did not survive correction for multiple comparisons [372]. Moreover, there appears to be shared heritability between BDs and MDD [28, 373], and longitudinal frontal cortical thinning might be a stable marker for familial vulnerability for depressive illness [374]. On the other hand, a twin study did not find any association between genetic liability for BD and reduced cortical thickness, but rather that unique environmental factors related to BD were associated with cortical thinning in right frontal, limbic, and occipital cortices [375]. Thus, although several studies suggest that reduced frontotemporal cortical thickness is associated with increased risk for BDs and might represent a vulnerability marker, further longitudinal studies are needed to fully clarify the temporal profile of the cortical thinning.
5.1.3 Association between VEP plasticity and cortical thinning

In Paper III we used plasticity of the VEP as an index of cortical synaptic function. We found that plasticity of the N1 amplitude at baseline was significantly associated with subsequent thinning in widespread cortical regions across patients with BD type II and HCs, indicating greater thinning over time in subjects with less N1 plasticity. The results are in line with previous cross-sectional studies demonstrating an association between cortical thickness and measures of LTP-like cortical plasticity [376, 377]. While detailed histological examinations are required to fully clarify the cellular alterations underlying cortical thinning in BD type II and in normal aging, loss of dysfunctional synapses is one potential neurobiological substrate.

Here, the results and how they contribute to greater understanding of cortical abnormalities in BD type II will be discussed by addressing the following questions:

1) Can loss of dysfunctional synapses contribute to cortical thinning?; and

2) Is synaptic loss a possible mechanism for cortical thinning in BD type II?

Can loss of dysfunctional synapses contribute to cortical thinning?

In the mammalian brain, the number of synapses correlates positively with thickness of the cortex and synaptic volume constitutes ~10% of total cortical volume [273, 274, 378]. A substantial loss of synapses might therefore contribute to cortical thinning measurable with MRI. In normal adult human aging, MRI studies indicate an annual thinning of approximately 0.5% in most cortical regions [259]. Healthy aging is most likely not associated with substantial loss of neurons [379, 380] and previous studies indicate a reduction in synaptic density and number in brains of older individuals [144, 259, 265, 268, 381, 382]. One previous study found that neurologically normal older individuals had 40-50% fewer dendritic synapses in prefrontal and occipital cortices than younger individuals, indicating that age-related synapse loss is considerable [268]. Moreover, excitatory and highly plastic glutamatergic synapses may be particularly vulnerable to degeneration during aging [383-385]. Although the precise molecular mechanisms underlying age-related synaptic loss are not fully characterized, previous studies in aged individuals found reduced expression of genes implicated in synaptic plasticity and function, and a gradually decreased expression of synapse-related proteins [386-389]. Signal transmission across synapses is energetically expensive and synapses are therefore vulnerable to degeneration when neuronal function is compromised [390, 391]. Furthermore, studies have linked aging to deficits in LTP-like plasticity and to facilitated LTD-like plasticity, which are associated with loss of synapses [144-148].
The associations between LTP-like cortical plasticity and cortical thinning in Paper III provide additional, albeit indirect, support for the hypothesis that loss of dysfunctional synapses contributes to cortical thinning. We found that plasticity of the N1 VEP amplitude at baseline was significantly negatively associated with thinning in widespread cortical regions across HCs and individuals with BD type II. The association remained significant in right medial occipital, parietal, temporal, and posterior cingulate cortices after exclusion of two patient outliers from the analyses. Furthermore, when the analysis was run in HCs alone, there was a significant negative association between N1 plasticity and cortical thinning in a left posteromedial cluster, comprising the cuneus, precuneus, and posterior cingulate cortex.

The neural sources of the VEP components remain to be fully clarified, yet studies suggest that the N1 component reflects postsynaptic potentials in both primary and extrastriate visual cortices of the medial occipital lobe [244, 392-394]. Thus, the medial occipital cortical regions showing the strongest association between N1 plasticity and cortical thinning may include neural generators underlying the N1 amplitude. Although speculative, this observation suggests that dysfunctional synapses in medial occipital cortices, as indicated by reduced N1 plasticity, may be prone to elimination, which in turn might contribute to cortical thinning. Plasticity of the N1 component also correlated negatively with thinning rate in medial parietal cortices, including the precuneus and posterior cingulate cortex, which overlap with the posterior node of the default mode network (DMN). The brain regions comprising the DMN are characterized by high metabolism and aerobic glycolysis, believed to reflect high levels of synaptic turnover and remodeling [395-399]. These cortical regions might be especially sensitive to synaptic function alterations and thus show stronger associations between thinning and VEP plasticity than other cortices.

Although further studies are needed to fully clarify the relationships between synaptic deficits, VEP plasticity, synapse loss, and cortical thinning, the results of previous studies and the present thesis suggest that aging- and synaptic dysfunction-related loss of synapses might be one neural substrate for thinning of the cerebral cortex.

**Is synaptic loss a possible mechanism for cortical thinning in BD type II?**

Genetic risk variants implicate synaptic function and plasticity in the pathophysiology of mood disorders [322, 400-402]. Furthermore, patients with BDs and MDD demonstrate altered expression of synapse-related genes and decreased levels of synaptic signaling proteins [403-405]. Only a few neuropathological studies have examined cortical synapse number in BD type II and other mood disorders, yet the available studies support reductions in synapses in regions...
implicated in mood regulation. One study found reduced number of spines per dendrite (~26% reduction) and dendritic spine density (~11% decrease) in dlPFC of individuals with BDs (the BD subtype was not specified) compared to HCs [269]. Another study reported lower spine density and dendrite length in PFC of patients with BD type I, however, no changes were detected in patients treated with lithium [406]. Furthermore, a third study observed ~50% fewer synapses in dlPFC of patients with MDD compared to control subjects [270]. In this study, patients demonstrated decreased expression of several genes related to synaptic function, while expression of the transcription factor GATA1 was increased. Overexpression of GATA1 in cultured prefrontal cortical neurons resulted in decreased expression of synapse-related genes, loss of spines and dendrites, and produced depression-like behavior in rodents [270].

A recent study found repression of the regulatory unit of early growth response gene 3 (EGR3), which is part of the immediate early gene (IEG) family, in individuals with BDs [407]. These genes become activated in the brain in response to environmental stimuli, such as stress, and are believed to translate environmental factors to long-term brain changes through various neurobiological processes, including synaptic plasticity [270, 407]. Results from previous studies indicate that stress exposure causes atrophy of dendrites and loss of spines in the hippocampus [408-410] and in the PFC [176, 411-413]. In stress susceptible-mice increased ERG3 transcription induced dendritic atrophy and most likely synapse loss at medium spiny neurons in the nucleus accumbens [414]. Taken together, these studies suggest that loss of synapses might contribute to cortical thinning in mood disorders [9, 260], yet histological studies are needed to confirm this hypothesis in BD type II.

### 5.1.4 Is bipolar disorder type II a neuroprogressive disorder and are the cortical changes reversible?

The term “neuroprogression” refers to progressive neuropathological changes that occur in parallel to functional and clinical deterioration in neuropsychiatric illnesses [54]. Whether BDs are neuroprogressive disorders remains a matter of debate [55, 415]. There is a scarcity of longitudinal studies examining brain structure and function in BDs in general and in BD type II in particular. Paper II of this thesis found increased temporal and ventromedial cortical thinning in patients who experienced many depressive episodes between baseline and follow-up examinations. These findings support progressive cortical changes in BD type II and suggest that they may be related to mood episode effects. There are only three other published longitudinal studies of cortical thickness in BD. The first study found increased cortical thinning in the dlPFC of individuals with BD type I that had at least one manic episode during a follow-
up period of six years [358]. The second study examined cortical thinning over one year in patients with first-episode psychosis (where only a subgroup had BD) and in HCs and found no group difference [416]. The third study assessed cortical structural changes over time in BD twins and HCs and detected no differences [417].

Other longitudinal studies reported increased rates of GMV loss in adolescents [418] and adults [359] with BD type I, and one study found frontal and temporal GMV loss only in patients that experienced a mood episode during follow-up [355]. These findings are consistent with some cross-sectional reports, which demonstrated associations between brain structure alterations in BD and number of previous mood episodes [101, 352-357]. In contrast, two longitudinal studies found no GMV changes in BD patients with first episode psychosis [419, 420].

Another important question is whether the brain alterations in BD type II and other mood disorders are reversible. A recent MDD study found impaired LTP-like plasticity in the motor cortex during an acute depressive episode and normalization after remission, indicating that cortical plasticity impairments might be reversible, at least in MDD [341]. However, the potential for normalization of LTP-like plasticity in BD type II could be more limited since VEP plasticity was impaired also in the euthymic patients of Paper I. Another study reported that remission in patients in MDD was associated with increased thickness in frontotemporal cortices, while non-remitters showed thickness reductions in the same regions [421]. Other works suggest that cortical abnormalities can be reversed by treatments used in BDs. Lithium can increase GMV in the hippocampus and the cortex and at least reduce progression of cognitive dysfunction [422-424]. The rapid-acting antidepressants scopolamine and ketamine may increase synapse number and function in prefrontal cortices [208, 425, 426] and could potentially reverse cortical thinning.

To summarize, there is emerging evidence for neuroprogression in BDs [427, 428] and that mood episodes may contribute to the cortical alterations [415]. However, the sample sizes of longitudinal studies conducted to date are limited and most studies focused on BD type I. The results of the present thesis indicate that cortical thickness and plasticity abnormalities in adults with BD type II are stable features and that a high burden of mood episodes might be associated with further progression of the cortical changes. More longitudinal research is needed to confirm these findings and to clarify whether the cortical abnormalities of BD type II can be reversed.
5.2 Methodological considerations

There are several limitations that need to be taken into consideration when interpreting the findings of the present thesis.

5.2.1 Medication effects on cortical structure and plasticity

The individuals with BD type II were included in the present study regardless of medication status, and most patients were medicated at follow-up. The potential for confounding effects of medication is common in neuroimaging studies in psychiatry and there are relatively few studies of unmedicated patient groups. Patients oftentimes have severe symptoms that require acute treatment and that prohibit cessation of psychotropic drug treatment before study participation.

Medication effects could potentially contribute to the cortical changes reported in this thesis. However, it is unlikely that the medications alone would account for the findings due to several reasons. In Paper I we found reduced N1 and P1-N1 plasticity in patients relative to controls. There was no significant association with medication use (controls vs. unmedicated vs. medicated patients) and N1 plasticity, however, there was a significant association with P1-N1 plasticity. Post-hoc analyses showed reduced plasticity in medicated patients compared to controls, but not in unmedicated patients. However, there was no significant difference in P1-N1 plasticity between unmedicated and medicated patients, as shown in Table S4 of Paper I. The lack of significant difference in plasticity between controls and unmedicated patients probably results from the small sample size of this patient group (n=7). Furthermore, previous studies found a trend towards a less severe impairment in VEP plasticity in medicated than in unmedicated individuals with BD type II and that sertraline and fluoxetine, which were also used by patients of the present thesis, increase VEP plasticity in HCs [251] and synaptic plasticity in the adult rat visual cortex [429]. Thus, it seems unlikely that medication use underlies the VEP plasticity impairments in BD type II found in this thesis.

In Paper II, patients had increased rate of cortical thinning in the left temporal cortex, and there was no effect of current psychotropic medication use, antidepressant use, use of mood stabilizers, or use of antipsychotics on thinning rate in this region. There is only limited research in medication effects on brain structure and function in BDs through repeated assessments before and after specific medication regimes and most studies have explored medication effects in secondary analyses. A recent cross-sectional study found thinner prefrontal cortices in patients with BD type I compared to HCs, and that lithium treatment was associated with
increased bilateral frontal cortical thickness compared to valproate treatment [430]. Furthermore, patients using lithium had no significant differences in frontal cortical thickness compared to HCs [430]. A majority of previous studies assessing medication effects in BD were functional activation studies in youths, and despite several limitations such as short treatment periods and lack of placebo groups, these studies generally showed that treatment with mood stabilizers and antipsychotic agents (lamotrigine [431-435], risperidone [436], divalproex [436], and ziprasidone [437]) was associated with normalization of activation in tasks of emotional and cognitive processing [438]. To conclude, most previous studies found either no significant association with medication status or reported that medication appeared to have a normalizing effect on brain changes and it is unlikely that psychotropic drug treatment underlies the cortical thinning observed in subjects with BD type II in Paper II.

5.2.2 Sample size and generalizability

An important limitation of the present thesis is the modest sample size, particularly of the longitudinal samples. In order to increase sample size for cross-sectional analyses of cortical abnormalities and clinical variables we recruited additional patients and controls at follow-up. Nevertheless, these assessments might be underpowered and larger studies are needed to further clarify potential associations with clinical variables on cortical structure and plasticity.

Although the sample size of the present thesis is modest, we believe that the patient sample is representative for subjects with BD type II treated at outpatient clinics in the Oslo area because they were recruited from five different outpatient clinics and had varying degrees of illness severity. Furthermore, key sociodemographic and clinical characteristics of the patients, such as sex distribution, frequency of comorbid anxiety, age at onset of first affective symptoms that affected functioning, and disability benefit recipiency rate are largely consistent with findings in previous studies [23, 439-442].

Attrition is one of the major problems in longitudinal research, and generalizability of findings might suffer if subjects who drop out differ from those who complete follow-up examinations. However, there was no difference in plasticity of the C1, P1, N1 or P1-N1 amplitudes at baseline between subjects who dropped out and subjects who completed follow-up examinations, in both the patient and the control group (not reported in Paper I). Furthermore, there was no difference in cortical thickness in the left and right prefrontal and the left temporal clusters showing group differences at baseline between subjects who dropped out and subjects who completed follow-up examinations, in the total sample, in patients and in the control group (not reported in Paper
Thus, there are no indications of differences in cortical plasticity or structure in participants who dropped out compared to those who completed follow-up examinations. Nevertheless, since clinical trajectory for participants who did not show up for follow-up examinations remains unknown, we cannot definitely rule out that they do not represent a specific subgroup of the population, thereby reducing generalizability of results.

5.2.3 Comparison groups

The present study did not include comparison groups, e.g., individuals with BD type I or MDD. Accordingly, this thesis cannot answer whether the observed alterations in cortical thickness and plasticity, and the associations with depressive episodes, are specific for BD type II or neurobiological characteristics shared across mood disorders.

5.2.4 Issues related to data interpretation

VEP plasticity paradigm

An inherit limitation of the VEP plasticity paradigm in mood disorder research is induction and recording of LTP-like plasticity in early visual cortices, rather than in brain regions thought to be implicated in BDs pathophysiology, as discussed in Section 5.1.1. In addition, it remains to be clarified whether VEP plasticity reflects LTP-like plasticity in other cortical regions. Moreover, EEG-data was recorded from a limited number of electrodes. The use of high-density EEG or magnetoencephalography in future studies may aid investigations of cortical plasticity dysfunction across the cortical mantle, aid in localization of neural generators of evoked potentials, and increase our understanding of how modulation of cortical excitability might change neural circuitry in mood disorders. Furthermore, it is well established that the VEP amplitudes reflect postsynaptic potentials created by assemblies of pyramidal cells in the visual cortex [241], yet the precise neural generators of the individual VEP amplitudes remain to be fully clarified [244, 392-394]. This limits the conclusions that can be drawn from association analyses of VEP plasticity and cortical thinning and complicates the interpretation of the plasticity effects involving specific VEP components. Furthermore, detailed studies in rodents indicate that plasticity of the VEP reflects cortical processes closely related to LTP, as discussed in Section 1.4.2. However, one study suggests that other mechanisms, such as selective disinhibition of cortical circuitry, might play a role in VEP amplitude modulation [443]. More research is therefore needed to fully clarify the exact mechanisms that subserve VEP plasticity in humans.
Cortical thickness analyses

The thickness of the cortex is measured by automatic detection of signal intensity and contrast differences between grey and white matter in MRI images [287]. Several processes may influence the detection of this tissue boundary. For example, the white/grey tissue boundary can be blurred and therefore moved outward in regions high in intracortical myelin, resulting in apparent decreases in cortical thickness estimates [444]. Thus, apparent cortical thickness decreases could partly reflect increased myelination of neuronal axons, rather than true reductions of grey matter components. However, a previous study showed that cortical thickness reductions in BDs primarily occurred in deep heavily myelinated cortical tissue, making it unlikely that increased intracortical myelination would account for the apparent thickness reductions [445]. In addition, regional variations in cortical iron and water content might affect cortical thickness estimates obtained from T1-weighted MRI images [446], and group differences of these molecules might theoretically influence the results. Further progress in MRI technology will advance the search for biological mechanisms underlying cortical thickness changes in BDs and other mood disorders [447].

Longitudinal neuroimaging is increasingly used to assess cortical changes in brain disease and in normal aging. Previous studies have often employed tools designed for cross-sectional analyses, where a single common anatomical reference template is often used to guide tissue segmentation [293]. A potential pitfall is therefore that noise from inter-individual morphological differences might overshadow longitudinal anatomical within-subject changes. The recently developed longitudinal image processing tools applied in this study has enabled reduction of within-subject variability by transferring information across time, thus informing processing from later time points with knowledge from earlier scans [293]. These tools have increased sensitivity and reliability of measurements allowing for detection of subtle brain changes even with relatively small sample sizes and short follow-up periods [293].

The specification of an appropriate threshold for statistical maps is important for structural MRI data analyses [448]. Freesurfer constructs models of the cortical surface creating a mesh of triangles which are tessellated into approximately 150,000 vertices for each hemisphere [285]; thus, running statistical analyses for each vertex creates a massive multiple comparisons problem. In Paper II we corrected for multiple comparisons using Monte Carlo simulations implemented in Freesurfer. This method performs simulations under the null hypothesis and calculates the distribution of maximum cluster size in randomly generated maps, thereby determining the cluster-wise $p$-values [297]. Conservative statistical thresholding attempting to
diminish Type I errors (i.e., false findings resulting from noise rather than true effects) might come at the cost of increased Type II errors (i.e., missing true effects), and a bias towards studying large rather than small effects. As neuroimaging in mood disorders currently is in an exploratory phase, it can be argued that it is more important not to dismiss possible true effects, which should be explored further, than it is to avoid reporting false findings, as they are not likely to be replicated [449]. With this in mind we reported both corrected and uncorrected results. Moreover, a recent study indicated modest inflation of false positive rates for surface-based cortical thickness analyses using parametric clusterwise correction for multiple comparisons [450]. Based on this finding, we used permutation-based non-parametric tests (Permutation Analyses of Linear Models - PALM) [298] for assessing statistical significance in Paper III.

**Association between VEP plasticity and cortical thinning**

An important limitation in Paper III is that the interpretation of results must remain speculative. VEP plasticity and cortical thickness changes are both measures where the precise underlying neural substrates are not completely understood. The association between these measures therefore needs to be interpreted with caution. However, the results support our *a priori* hypothesis that neurobiological processes underlying VEP plasticity and cortical thinning are associated, possibly pointing to the involvement of cortical synapses. Nonetheless, the association between VEP plasticity and cortical thinning must be replicated in larger samples.

### 5.2.5 Study design

There are few prospective studies of BD type II neurobiology and the prospective design is a strength of the current thesis. However, the direction of the relationship between mood episodes and brain changes remains to be elucidated. Furthermore, the number of mood episodes the patients experienced between baseline and follow-up were assessed retrospectively and is therefore prone to recall bias [451]. Lastly, we do not have reliable information about duration of mood episodes, and total time spent in depression or hypomania might be a better correlate of disease severity than number of mood episodes.

### 5.3 Summary and implications for future research

The current thesis examined cortical plasticity and thickness in BD type II, and provides the first longitudinal evidence indicating that impaired LTP-like plasticity and reduced frontotemporal cortical thickness might be stable traits in adults with the disorder. Furthermore,
the results indicate that depressive episodes are associated with further deterioration of the cortical alterations, and that cortical plasticity and thickness changes might be associated processes. These findings support a growing body of evidence suggesting that BDs are associated with impaired synaptic function and plasticity, and that loss of dysfunctional synapses might contribute to cortical thickness reductions. However, the results need to be replicated in larger samples, and future studies should include patients with other mood disorders to examine the specificity of the findings. Furthermore, future studies are needed to elucidate the precise mechanisms underlying impaired VEP plasticity and cortical thinning in BD type II.

In Paper I there was no significant association between salivary cortisol and VEP plasticity in BD type II. However, other stress-related indices, such as hair cortisol, heart rate variability or alpha-amylase levels, might be associated with VEP plasticity and should be examined in future studies. Moreover, more research is needed to investigate whether VEP plasticity correlates with LTP-like cortical plasticity induced by other modalities, such as LTP-like plasticity in the auditory cortex and TMS-induced plasticity in the motor cortex and in the dIPFC.

Paper II demonstrated regionally increased rate of cortical thinning in BD type II, however, the underlying cellular processes remain unknown. The results of Paper III support the hypothesis that loss of dysfunctional synapses might be involved in increased cortical thinning in mood disorders. Recent advances in high-resolution MRI acquisition and modeling of grey matter microstructure have facilitated endeavors to bridge the gap between neuroimaging and histopathological analyses, by attempting to estimate gray matter microstructure in vivo [452-455]. For instance, modeling of grey and white matter microstructural properties using neurite orientation dispersion and density imaging (NODDI) [452] could advance our understanding of the processes underlying cortical thinning in BD type II. To date, one study has examined patients with BD type I using such approaches, and reported reduced NODDI-based neuritic density in bilateral temporal structures compared to HCs, although the results did not survive corrections for brain-wide analyses [456].

Furthermore, recent developments in molecular brain imaging, such as imaging of glutamate transmission, could provide opportunities for more precise assessments of synaptic function and mechanisms related to treatment effects in BDs [457-460]. In addition, novel techniques to visualize and analyze immunohistochemically stained slides of brain tissue have improved the quantification of cortical neuron density, neuron size, and cortical layer thickness [461], and
the study of circuitry and spatial interaction between cells in brain samples could contribute to increased understanding of cortical alterations in mood disorders [462, 463].

Whether BD type II is a neuroprogressive disease remains to be clarified, as discussed in Section 5.1.4. Future studies should examine longitudinal brain alterations in patients with similar illness severity at baseline, in order to assess whether progressive changes occur in all patients, or only in subgroups characterized by specific illness trajectories. To further evaluate the effect of mood episodes on VEP plasticity and cortical thinning, future studies could examine patients at admission for an acute mood episode and after remission, although the results of the current thesis suggest that one mood episode might not be sufficient to detect cortical thickness changes. Furthermore, appropriately designed studies are needed to examine whether cortical changes in BD type II are reversible, e.g., by psychotropic drugs. Moreover, human induced pluripotent stem cells could be used to examine intra-individual correlations between in vivo and in vitro indices of synaptic function and plasticity, whether synaptic impairments are associated with known genetic risk variants for BD, and whether they can be reversed by pharmacotherapy.

Future studies should also consider whether other potential confounders, such as sleep, might contribute to cortical changes observed in the current and previous work. Sleep disturbances are prevalent in BDs [464], are associated with functional and structural cortical changes in healthy individuals, including GMV reductions in prefrontal cortices [465], and thus warrant further investigation in this context.

Furthermore, the functional significance of impaired VEP plasticity and thinning of the cerebral cortex in specific regions remains to be elucidated. Finally, advanced multimodal analyses of combined functional and structural cortical measures may provide important insights into the likely neurobiological heterogeneity of BD type II and could be used for patient stratification and treatment individualization.
6. Concluding thoughts

The human brain holds a remarkable capacity for adapting to changing demands of the surroundings. The mechanisms underlying flexibility in information transfer across synaptic connections are immensely complex and depend on the activity of more than 1500 postsynaptic proteins and hundreds more that operate presynaptically. It has been hypothesized that the positive selection of synaptic proteins has resulted in the evolution of complex human cognition and behavior at the cost of increased vulnerability for psychiatric conditions [466, 467].

Preclinical studies suggest that BD type II and other mood disorders might result from impairments of synaptic function and plasticity and loss of synapses in neural circuits underlying mood and emotion regulation. However, mainly due to a lack of non-invasive methods, there has been a paucity of human in vivo evidence for this hypothesis. The results from this longitudinal study support impairments of LTP-like plasticity in the cerebral cortex of patients with BD type II, cortical thinning in regions implicated in mood regulation, and indicate that these changes might be stable traits in adults with BD type II. Furthermore, depressive episodes may cause further deterioration of these cortical alterations. Finally, LTP-like cortical plasticity at baseline was associated with longitudinal cortical thinning, which, although preliminary, support the hypothesis that loss of dysfunctional synapses might contribute to cortical thickness reductions observed in BD type II and other mood disorders.

The findings of this thesis highlight the importance of early intervention and prevention of mood episodes in BD type II. The high level of illness burden and disability associated with BDs underscore the urgent need for new and more effective treatment options. An important premise for therapeutic headway is increased understanding of pathophysiological pathways involved in development and progression of illness. Increased insight could lead to mechanism-based reclassification and treatments of psychiatric disorders and improved patient outcomes.
7. References


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Longitudinal and cross-sectional investigations of long-term potentiation-like cortical plasticity in bipolar disorder type II and healthy individuals

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Abstract
Visual evoked potential (VEP) plasticity is a promising assay for noninvasive examination of long-term potentiation (LTP)-like synaptic processes in the cerebral cortex. We conducted longitudinal and cross-sectional investigations of VEP plasticity in controls and individuals with bipolar disorder (BD) type II. VEP plasticity was assessed at baseline, as described previously (Elvsåshagen et al. Biol Psychiatry 2012), and 2.2 years later, at follow-up. The longitudinal sample with VEP data from both time points comprised 29 controls and 16 patients. VEP data were available from 13 additional patients at follow-up (total n = 58). VEPs were evoked by checkerboard reversals in two premodulation blocks before and six blocks after a plasticity-inducing block of prolonged (10 min) visual stimulation. VEP plasticity was computed by subtracting premodulation VEP amplitudes from postmodulation amplitudes. Saliva samples for cortisol analysis were collected immediately after awakening in the morning, 30 min later, and at 12:30 PM, at follow-up. We found reduced VEP plasticity in BD type II, that impaired plasticity was present in the euthymic phases of the illness, and that VEP plasticity correlated negatively with depression severity. There was a positive association between VEP plasticity and saliva cortisol in controls, possibly reflecting an inverted U-shaped relationship between cortisol and synaptic plasticity. VEP plasticity exhibited moderate temporal stability over a period of 2.2 years. The present study provides additional evidence for impaired LTP-like cortical plasticity in BD type II. VEP plasticity is an accessible method, which may help elucidate the pathophysiological and clinical significance of synaptic dysfunction in psychiatric disorders.

Introduction
Bipolar disorder (BD) type I and II affect 2–3 % of the population and can lead to marked impairments in social and occupational functioning1–3. The estimated heritability of BD is ~ 0.74,5, yet its precise pathophysiological basis remains unknown. Consequently, current therapeutic options may not target fundamental illness processes and remain insufficient for a substantial number of patients6,7. The clarification of central pathophysiological mechanisms is therefore a critical step toward improved outcomes in BD.

Synaptic dysfunction is one of the leading candidate mechanisms across psychiatric illnesses6–13. In particular, preclinical studies and genetic investigations have implicated synaptic plasticity in the etiology and treatment of BD, major depressive disorder (MDD), autism spectrum disorder, and schizophrenia13–23. Despite these findings, there is a paucity of clinical evidence supporting synaptic dysfunction.
dysfunction in psychiatric disorders, mainly due to a lack of methods for noninvasive measurements of synaptic function and plasticity in humans. However, electroencephalography (EEG)-based measurement of visual cortex plasticity has in recent years emerged as a promising assay for in vivo assessment of synaptic function and plasticity. Previous studies showed that repeated visual stimulation-induced increases of the human visual evoked potential (VEP), i.e., an EEG signal that primarily reflects postsynaptic potentials in the visual cortex. Further investigations found that VEP plasticity was reduced in BD type II, MDD, and schizophrenia. Although the precise neural substrates for VEP plasticity in humans remain to be clarified, detailed studies in rodents showed that VEP increases induced by repetitive visual stimulation is long-lasting, stimulus-specific, and depends on synaptic N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors and protein kinase M. These are all core features of long-term potentiation (LTP), which is the best characterized form of synaptic plasticity. Together, these results indicate that VEP plasticity is reduced and may reflect impairments of cortical LTP-like synaptic processes, in BD type II, MDD, and schizophrenia.

Despite these promising findings, more research is needed to clarify the mechanisms, translational potential, and clinical utility of VEP plasticity in BD and other psychiatric illnesses. Impaired VEP plasticity across psychiatric illnesses may suggest that nonspecific mechanisms such as stress- and cortisol-related synaptic dysfunction could underlie the plasticity reductions. Moreover, the relationships between VEP plasticity and clinical characteristics of psychiatric illnesses remain to be clarified. In addition, there are to our knowledge no longitudinal studies of VEP plasticity and its temporal stability in healthy volunteers and patient groups remains unknown.

We previously found plasticity of the VEP in healthy controls and reduced plasticity in BD type II. Here, we conducted longitudinal and cross-sectional investigations of VEP plasticity in individuals with BD type II and controls with the following main aims: (1) to test the reproducibility of impaired VEP plasticity in BD type II and to assess the relationship between mood state and plasticity, (2) to examine the relationship between saliva cortisol and VEP plasticity in controls and BD type II, and (3) to examine the temporal stability of VEP plasticity.

Methods and materials

Participants and clinical examinations

We assessed VEP plasticity at Oslo University Hospital in 40 controls and 26 individuals with BD type II at baseline, as described previously. At follow-up, on average 2.2 years later, 33 of the controls and 18 of the patients again underwent the VEP plasticity examinations. Two patients and four controls were excluded from the analyses owing to technical issues during EEG recording and insufficient data quality, thus the longitudinal sample comprised 29 controls and 16 patients. Moreover, 16 additional patients and one new control were included at follow-up to further assess the relationship between VEP plasticity and saliva cortisol and mood state. Owing to technical issues and insufficient data quality, three of the new patients and the new control were excluded from the analyses. Thus, the cross-sectional patient sample at follow-up included 29 participants. In controls, the longitudinal sample and the cross-sectional sample at follow-up were identical and comprised 29 individuals.

The patients were recruited from psychiatric outpatient clinics in the Oslo area. Clinical examinations at baseline and follow-up were carried out by senior psychiatrists (i.e., authors EB, BB, and UFM) at a university department specializing in the evaluation and treatment of mood disorders. Axis I diagnoses and psychiatric comorbidities were determined with the Mini-International Neuropsychiatric Interview, DSM-IV criteria version 5.0. Alcohol and drug use were assessed with the Alcohol Use Scale and the Drug Use Scale, respectively. Mood state was assessed at the day of EEG recording for the large majority of participants and within 3 days of the recording for all participants. Assessments were carried out by trained physicians (i.e., authors EB, BB, UFM, and TE) using the Montgomery–Asberg Depression Rating Scale (MADRS) and the Young Mania Rating Scale (YMRS). These physicians underwent a day course of MADRS and YMRS prior to the present study, which included estimation of their intraclass correlation coefficient (ICC); all ICCs were > 0.8.

Controls with no previous or current psychiatric illness were recruited through local advertising and underwent a full examination similar to that of the patients at baseline and follow-up. The exclusion criteria for all subjects were: age below 18 or above 50 years, history of neurological or other severe chronic somatic disorder, and pregnancy. One patient had experienced a mild head injury with loss of consciousness for > 1 min. However, the patient did not have any clinical or magnetic resonance imaging-detectable cerebral sequela and was included in the study. Otherwise, no participant reported head injury with loss of consciousness for > 1 min. All subjects had normal or corrected-to-normal visual acuity. The Regional Ethical Committee of South-Eastern Norway approved the study, and all subjects provided written informed consent to participate.
Experimental paradigm

The experimental paradigm described by Normann et al.28, was used at baseline and follow-up. VEPs were evoked by checkerboard reversals (check size = 0.5°; 2 reversals/sec) in two premodulation blocks before and six blocks after a plasticity-inducing modulation block (Fig. 1). In each pre- and postmodulation block, 40 checkerboard reversals were presented within 20 s. In the modulation block, VEPs were evoked by checkerboard reversals (check size = 0.5°; 2 reversals/sec) for 10 min. The premodulation blocks were initiated 2 and 8 min after the start of the experiment, and the modulation block was initiated 2 min after the last premodulation block. Then, the postmodulation blocks were performed 2, 8, 12, 18, 22, and 28 min after the end of the modulation block. A gray screen was displayed between checkerboard stimulation. Participants were instructed to focus on a filled red circle (0.1°) in the center of the screen during the experiment. They were monitored throughout the experiment to ensure that they followed instructions and maintained attention, and were allowed to listen to music. The visual stimuli were presented with E-Prime 1.1 (Psychology Software Tools, Sharpsburg, Pennsylvania) on a Samsung Syncmaster 2493HM LCD screen (Samsung Electronics Nordic AB, Oslo, Norway). To ensure high timing accuracy, a photodiode from the Black Box Toolkit® (Sheffield, UK) was used and VEP latencies were corrected accordingly.

Recording and analysis of the VEP

VEP plasticity was assessed using EEG data from the Oz electrode at both time points. The baseline examination also included mismatch negativity and oddball paradigms. Continuous EEG activity was therefore recorded from 15 monopolar silver/silver chloride electrodes for analyses of these paradigms at baseline. However, the follow-up examination only comprised the VEP plasticity paradigm and only three electrodes were therefore used (O1, Oz, and O2). All impedances were maintained below 5 kΩ and the ground and reference electrodes were attached to the forehead (AFz). Eye movements were recorded with bipolar electrodes placed at the sub- and supraorbital regions and at the lateral canthi of each eye. EEG activity was recorded at 250 Hz with an amplifier band-pass of 0.05–100 Hz. Offline EEG analysis was conducted with EEGLAB23, run on MATLAB 7.6.0. (MathWorks, Natick, Massachusetts). The EEG was first high-pass filtered at 1 Hz, and segmented into epochs starting 150 msec before and continuing 350 msec after the onset of each checkerboard reversal. All epochs containing eye movement-related activity were removed from analyses.

Epochs were then shortened (−50 to 350 msec) and baseline-corrected (−50 to 0 msec), and epochs with amplitudes exceeding ± 100 μV on any of the occipital channels (O1, Oz, O2) were rejected. The epoched EEG was finally low-pass filtered at 30 Hz and averaged to block-specific VEPs. Peak amplitudes and latencies for the C1, P1, N1, and the P1–N1 peak-to-peak amplitudes were obtained from the Oz electrode at the occipital head; amplitudes were measured relative to the 50 msec baseline.

Saliva collection and cortisol analysis

Saliva samples for cortisol analysis were collected using Salivette® Cortisol swabs (Sarstedt AG & Co, Nümbrecht, Germany) and analyzed with a Cortisol Saliva Luminescence Immunoassay (IBL International, Hamburg, Germany) according to the manufacturers’ instructions. Saliva samples were obtained the day after the VEP
Table 1  Demographic and clinical characteristics of the longitudinal and the cross-sectional samples of patients with bipolar disorder type II and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Longitudinal samplea</th>
<th></th>
<th>Cross-sectional sample at follow-up</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BD type II (n = 16)</td>
<td>Control group (n = 29)</td>
<td>BD type II (n = 29)</td>
<td>Control group (n = 29)</td>
</tr>
<tr>
<td>Age, years, mean (SD)</td>
<td>32.7 ± 7.5</td>
<td>33.1 ± 9.4</td>
<td>35.5 ± 7.9</td>
<td>35.6 ± 9.6</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>9 (56)</td>
<td>16 (55)</td>
<td>19 (66)</td>
<td>16 (55)</td>
</tr>
<tr>
<td>Education level, n (%)</td>
<td>0–10 years</td>
<td>2 (12.5)</td>
<td>0 (0)</td>
<td>2 (7)</td>
</tr>
<tr>
<td></td>
<td>11–13 years</td>
<td>4 (25)</td>
<td>3 (10)</td>
<td>8 (28)</td>
</tr>
<tr>
<td></td>
<td>14–17 years</td>
<td>8 (50)</td>
<td>11 (38)</td>
<td>9 (31)</td>
</tr>
<tr>
<td></td>
<td>17+ years</td>
<td>2 (12.5)</td>
<td>15 (52)</td>
<td>10 (34)</td>
</tr>
<tr>
<td>MADRS, mean (SD)</td>
<td>11.9 ± 6.5</td>
<td>1.3 ± 2.2</td>
<td>&lt;0.001</td>
<td>8.8 ± 6.8</td>
</tr>
<tr>
<td>YMRS, mean (SD)</td>
<td>3.0 ± 3.4</td>
<td>0.3 ± 0.8</td>
<td>&lt;0.001</td>
<td>2.4 ± 2.5</td>
</tr>
<tr>
<td>Euthymia (MADRS &lt; 11, YMRS &lt; 8)</td>
<td>10</td>
<td></td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Depression (MADRS &gt; 11)</td>
<td>5</td>
<td></td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Hypomania (YMRS ≥ 8)</td>
<td>1</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Medication, n (%)</td>
<td>Unmedicated</td>
<td>6 (38)</td>
<td>7 (24)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antidepressants</td>
<td>6b (38)</td>
<td>7c (24)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lamotrigine</td>
<td>6 (38)</td>
<td>16 (55)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lithium</td>
<td>1 (6)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quetiapine</td>
<td>1 (6)</td>
<td>3 (10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methylphenidate</td>
<td>0 (0)</td>
<td>n.a.</td>
<td>2 (7)</td>
</tr>
<tr>
<td></td>
<td>Duration of illness, years, mean (SD)</td>
<td>17.3 ± 8.1</td>
<td>n.a.</td>
<td>18.0 ± 7.3</td>
</tr>
<tr>
<td></td>
<td>Social phobia, n (%)</td>
<td>4 (25)</td>
<td>0 (0)</td>
<td>10 (34)</td>
</tr>
<tr>
<td></td>
<td>Panic disorder, n (%)</td>
<td>6 (38)</td>
<td>0 (0)</td>
<td>18 (62)</td>
</tr>
<tr>
<td></td>
<td>General anxiety disorder, n (%)</td>
<td>2 (13)</td>
<td>0 (0)</td>
<td>2 (7)</td>
</tr>
</tbody>
</table>

*Characteristics at baseline
bAntidepressants were escitalopram, citalopram, bupropion, mirtazapine, and fluoxetine
cAntidepressants were escitalopram, citalopram, bupropion, mirtazapine, venlafaxine, sertraline, and mianserin

Statistical analyses

All statistical analyses were conducted with SPSS version 24 for Windows (IBM Corp., Armonk, NY) and a two-tailed p value of < .05 was considered significant. VEP amplitudes from the two premodulation and from the six postmodulation recordings were averaged as premodulation and postmodulation VEP, respectively. To examine the effect of the modulation block on the VEP, the C1, P1, N1, and P1–N1 premodulation amplitudes were compared with the corresponding postmodulation amplitudes with repeated measures analysis of variance (ANOVA) in controls and patients separately. VEP plasticity was computed by subtracting premodulation VEP amplitudes from the corresponding postmodulation amplitudes.

Experiment at three times: immediately after awakening in the morning, 30 min after the first collection, and at 12:30 PM. Participants were instructed to not brush teeth and to refrain from physical activity, nicotine, and caffeine before saliva collection and to not eat or drink the last 30 min before the samples were obtained. Saliva cortisol was averaged across the three collections. We also computed the cortisol awakening response (saliva cortisol 30 min post awakening minus cortisol at awakening), as a previous study found that the cortisol awakening response was associated with transcranial magnetic stimulation (TMS)-induced motor cortex plasticity. Complete cortisol data were missing for one control and two individuals with BD type II.
Saliva cortisol was compared between groups using ANOVA. VEP plasticity scores were subjected to ANOVAs and analyses of covariance, after testing the assumption of homogeneity of regression slopes, to examine the effects of group, covarying for saliva cortisol, premodulation VEP amplitudes, and educational level (in the case of significant group differences for the latter variables) and the effect of medication (controls vs. unmedicated patients vs. medicated patients, Bonferroni corrected for the three contrasts). The relationships between VEP plasticity and mood state, saliva cortisol, and other clinical variables were assessed with Pearson correlation, Spearman’s rank correlation, and repeated measures ANOVA. The temporal stability of VEP plasticity was examined using Pearson correlation and ICC_{1,1}.

**Fig. 2 VEP plasticity of the longitudinal sample at follow-up.** a Grand average premodulation (blue) and postmodulation (red) VEP in controls (n = 29). The modulation block resulted in significant plasticity of the P1, N1, and P1–N1 amplitudes. b Grand average premodulation (blue) and postmodulation (red) VEP in patients with BD type II (n = 16). There was no significant P1, N1, or P1–N1 plasticity in the patient group. c P1–N1 plasticity was significantly reduced in patients with BD type II relative to controls. There was no significant group difference in C1 or N1 plasticity; however, there was a trend toward reduced P1 plasticity in patients (p = 0.07). The group difference in P1–N1 plasticity remained significant after controlling for cortisol, premodulation amplitude, and educational level. ***p < 0.001. Error bars represent the s.e.m. d Temporal stability of VEP plasticity in controls (n = 16) and patients with BD type II (n = 16). There was a significant positive correlation between baseline and follow-up P1 plasticity and e N1 plasticity, but not f P1–N1 plasticity in controls. g There was no significant correlation between baseline and follow-up P1 plasticity of h N1 plasticity, however, i there was a significant correlation for baseline and follow-up P1–N1 plasticity in patients with BD type II. VEP, visual evoked potential. BD, bipolar disorder.
Results

Demographic and clinical variables

Demographic and clinical variables for the longitudinal and the cross-sectional samples are shown in Table 1. There were no significant group differences in age or gender. In the longitudinal sample, controls had a higher educational level than patients; otherwise there were no significant group differences. Eighteen out of 29 patients were euthymic (MADRS score < 11 and YMRS score < 8), eight patients were depressed (MADRS score 12–23) and three patients were hypomanic (YMRS score 8) at follow-up examinations.

VEP plasticity of the longitudinal sample

VEP plasticity of the longitudinal sample at baseline

We previously reported significant VEP plasticity in healthy controls and impaired plasticity of the P1–N1 amplitude in individuals with BD type II at baseline (when the whole sample of 40 controls and 26 individuals with BD type II was analyzed)29. Here, we reran the VEP analyses for the longitudinal sample at baseline (n = 45) and found no significant group differences in the C1, P1, N1, or the P1–N1 amplitudes of the premodulation blocks (all p > 0.05). There was significant plasticity of the P1 (F_{1,28} = 8.36, p = 0.007), N1 (F_{1,28} = 4.88, p = 0.036), and P1–N1 (F_{1,28} = 34.95, p < 0.001) amplitudes in controls (Supplementary Figure 1A), but not in patients (Supplementary Figure 1B; all p > 0.05). Relative to controls, there was significantly reduced P1–N1 plasticity (F_{1,43} = 13.82, p = 0.001) and a trend towards reduced P1 plasticity that did not reach significance (p = 0.065) in patients (Supplementary Figure 1C), consistent with the previously published results for the whole sample (n = 66)29. There was a significant negative correlation of P1 and N1 amplitudes in controls (r = −0.64, p < 0.001) and in patients (r = −0.77, p < 0.001).

VEP plasticity of the longitudinal sample at follow-up

At follow-up, checkerboard reversal stimulation produced the expected VEP amplitudes at the premodulation blocks, with C1 at 88.1 ± 0.9 msec (mean ± s.e.m); P1 at 114.3 ± 0.8 msec, and N1 at 147.1 ± 1.9 msec in controls (Fig. 2a) and with C1 at 87.4 ± 1.6 msec, P1 at 114.3 ± 1.3 msec, and N1 at 151.0 ± 3.1 msec in patients (Fig. 2b). There were no differences between patients and controls in the latencies of the premodulation or postmodulation amplitude peaks (all p values > 0.05). P1 and N1 latencies of the postmodulation blocks were significantly increased relative to the premodulation latencies in controls (115.8 ± 0.8 msec vs. 114.3 ± 0.8 msec, F_{1,28} = 11.17; p = 0.002 and 150.5 ± 1.8 msec vs. 147.1 ± 1.9 msec, F_{1,28} = 32.31; p < 0.001, respectively). There were no significant latency changes in patients and no significant effect of group on changes in latencies from premodulation to postmodulation blocks (all p values > 0.05). Patients had significantly greater P1–N1 amplitude at the premodulation blocks than controls (F_{1,43} = 4.51; p = 0.04), whereas no significant group differences were found for the C1, P1, or the N1 premodulation amplitudes (all p > 0.05). There was significant plasticity of the P1 (F_{1,28} = 5.31, p = 0.03), N1 (F_{1,28} = 7.89, p = 0.009), and P1–N1 (F_{1,28} = 157.48, p < 0.001) amplitudes in controls, but not in patients (all p > 0.05). In controls, the P1–N1 plasticity effect was significant for all postmodulation blocks (postmodulation block 1: F_{1,28} = 124.25, p < 0.001, block 2: F_{1,28} = 16.13, p < 0.001, block 3: F_{1,28} = 13.25, p = 0.001, block 4: F_{1,28} = 5.10, p = 0.032, block 5: F_{1,28} = 15.43, p = 0.001, and block 6: F_{1,28} = 15.42, p = 0.001; thus five out of six blocks surviving Bonferroni correction, p < 0.05/6) (Figure S2A). There was a significant negative correlation between modulation of P1 and N1 amplitudes in controls (r = −0.72, p < 0.001) and in patients (r = −0.50, p = 0.047). There was significantly reduced P1–N1 plasticity (F_{1,43} = 16.26; p < 0.001) and a trend towards reduced P1 plasticity (F_{1,43} = 3.48; p = 0.07), in patients relative to controls (Fig. 2c). There was no significant group-salivary cortisol level interaction, group-premodulation amplitude interaction or group-educational level interaction for P1–N1 plasticity (all p’s > 0.1), and the group difference remained significant after adjusting for these covariates (F_{1,34} = 8.51, p = 0.006).

Temporal stability of VEP plasticity in controls and patients

In controls, there was a significant association between P1 plasticity at baseline and follow-up (r = 0.53, p = 0.003; ICC_{1,1} = 0.53; Fig. 2d). There was also a significant relationship between N1 plasticity at baseline and follow-up (r = 0.57, p = 0.011; ICC_{1,1} = 0.47; Fig. 2e), whereas no significant association was found for P1–N1 plasticity (r = 0.11, p = 0.57; ICC_{1,1} = 0.11; Fig. 2f), in controls. In patients, there were no significant associations for P1 or N1 plasticity (r = −0.27, p = 0.30; ICC_{1,1} = −0.27 and r = −0.03, p = 0.093; ICC_{1,1} = 0.03, respectively, Fig. 2g, h), yet a significant association for P1–N1 plasticity was found (r = 0.57, p = 0.022; ICC_{1,1} = 0.55; Fig. 2i), when the baseline and follow-up VEP plasticity results were compared.

Explorative longitudinal analyses

There was no significant effect of time and no significant group-time interaction effect on P1, N1, or P1–N1 plasticity (all p > 0.05). There was no significant effect of adding a psychotropic drug on VEP plasticity changes from baseline to follow-up (patients unmedicated at baseline and medicated at follow-up (n = 5) vs. other patients (n = 11) vs. controls (n = 29); all p > 0.05; see also Table S5 for details). There were no significant associations between P1, N1, or P1–N1 plasticity at baseline and...
number of depressive and hypomanic episodes between baseline and follow-up (all $p > 0.05$).

**VEP plasticity and saliva cortisol of the cross-sectional sample at follow-up**

**Saliva cortisol**

The saliva cortisol analyses showed the expected morning awakening response in patients and controls at follow-up (Fig. 3a); there was no significant group difference in the awakening response ($p = 0.37$). Saliva cortisol averaged across the three collections was higher in patients than controls ($F_{1,53} = 5.35, p = 0.025$) and saliva cortisol at 12:30 PM was significantly increased in patients ($F_{1,55} = 6.59, p = 0.013$).

**VEP plasticity and clinical variables**

In the cross-sectional sample at follow-up, controls (Fig. 2a) and patients (Fig. 4a) showed the expected C1 at 87.4 ± 7.7 msec, P1 at 114.2 ± 0.6 msec, and N1 at 149.8 ± 1.5 msec after the checkerboard reversal. Patients had significantly greater N1 ($F_{1,56} = 4.33, p = 0.04$) and P1–N1 ($F_{1,56} = 13.74, p < 0.001$) amplitudes at the premodulation blocks than controls. There were no significant differences in premodulation N1 (13.6 μV and 9.9 μV in unmedicated and medicated patients, respectively) or P1–N1 (16.7 μV and 14.5 μV in unmedicated and medicated patients) amplitudes between unmedicated and medicated patients (see also Tables S1 and S2 for details). Premodulation N1 amplitude was larger in unmedicated patients than in controls and there were no significant difference in N1 amplitude when medicated patients and controls were compared (Table S1). Premodulation P1–N1 amplitude was significantly increased in both unmedicated and medicated patients relative to controls (both $p < 0.05$; Table S2). No significant group differences were found for the C1 or P1 premodulation amplitudes. Patients had lower C1 amplitudes at the postmodulation blocks than premodulation ($F_{1,26} = 11.73, p = 0.002$); no P1, N1, or P1–N1 plasticity was found in the patient group (all $p > 0.32$); however, there was a significant effect of modulation on P1–N1 amplitude in the first postmodulation block ($F_{1,28} = 10.74, p = 0.003$; Figure S2B). There was no significant correlation between saline cortisol and clinical variables. There was a significant positive correlation between saliva cortisol and plasticity of the P1–N1 amplitude, indicating greater plasticity with higher cortisol levels. There was also a significant positive correlation between the cortisol awakening response and plasticity of the P1–N1 amplitude in controls.

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**Fig. 3 Saliva cortisol and VEP plasticity.**

(a) Saliva cortisol was collected the day after the VEP experiment at three times: immediately after awakening in the morning, 30 min after the first collection, and at 12:30 PM. Saliva cortisol was averaged across the three collections and was significantly increased in patients with BD type II relative to controls. (b) In controls, there was a significant positive correlation between saliva cortisol and plasticity of the P1–N1 amplitude, indicating greater plasticity with higher cortisol levels. There was also a significant positive correlation between the cortisol awakening response and plasticity of the P1–N1 amplitude in controls. VEP, visual evoked potential; BD, bipolar disorder.
between VEP plasticity and YMRS score in patients (all increasing cortisol levels. In patients, there was a trending $p = 0.44$, $N1$ plasticity (cortisol awakening response and $P1$ $p = 0.03$; Fig. 4c, d). There were no significant associations between VEP plasticity and YMRS score in patients (all $p > 0.05$; Figure S3). Next, we found decreased $N1$ ($F_{1,46} = 4.51, p = 0.04$) and $P1–N1$ ($F_{1,46} = 4.83, p = 0.03$) plasticity in the euthymic patients ($n = 19$) relative to controls (Fig. 4c), which remained significantly reduced after adjusting for saliva cortisol and premodulation amplitudes (all $p < 0.05$). There were no significant differences in VEP plasticity between patients with and without panic disorder or social phobia (all $p > 0.05$).

**VEP plasticity and saliva cortisol**

In controls, there was a positive association between averaged saliva cortisol and $P1–N1$ plasticity ($r = 0.43, p = 0.023$; Fig. 3b) and a positive association between the cortisol awakening response and $P1–N1$ plasticity ($r = 0.44, p = 0.018$; Fig. 3c), indicating greater plasticity with increasing cortisol levels. In patients, there was a trending positive correlation between averaged saliva cortisol and $P1–N1$ plasticity that did not reach statistical significance ($r = 0.36, p = 0.037$).

**Discussion**

Plasticity of the VEP is a promising assay for non-invasive examination of cortical LTP-like synaptic processes. In the present study of VEP plasticity in individuals with BD type II and controls, there were three main findings. First, we reproduced impaired VEP plasticity in BD type II at the follow-up examinations. We also found that VEP plasticity was reduced in euthymic patients and was negatively correlated with depression severity. Second, we showed that saliva cortisol was increased in BD type II, that VEP plasticity remained impaired in patients after controlling for saliva cortisol, and that saliva cortisol was positively correlated with plasticity of the VEP in controls. Finally, plasticity of the $P1$ and $N1$ components of the VEP, but not the $P1–N1$ component, exhibited moderate temporal stability in healthy individuals when baseline and follow-up examinations were compared.

Although its precise neural basis remains unknown, BD has been conceptualized as a genetically influenced disorder of synaptic function and plasticity in limbic-cortical neural networks involving the amygdala, hippocampus,
between VEP plasticity and YMRS score in patients \( p = 0.44 \), with BD type II \( \Delta N1 \) plasticity \( \Delta \text{cortisol awakening response and P1} \), and that saliva cortisol was positively correlated with hypomania severity and VEP plasticity. Yet, the hypomania symptoms of the patients were generally mild and further studies are needed to clarify the relationship between hypomania and VEP plasticity.

Another finding of the present study was increased saliva cortisol in BD type II. This observation is consistent with two recent meta-analysis, which found elevated saliva and blood cortisol in BD, particularly in euthymic and manic patients \( p < 0.05 \). These studies involved individuals with BD type I or mixed samples of patients with BD type I or II \( p < 0.05 \), and we are not aware of any previous cortisol study that has been limited to BD type II.

The effects of glucocorticoids on synaptic function and plasticity oftentimes follow an inverted U-shaped curve \( 37,38 \). At low and high levels, glucocorticoids can impair synaptic function and plasticity, whereas normal glucocorticoid concentrations facilitate synaptic plasticity processes, such as LTP. These synaptic corticosteroid effects are likely mediated by both non-genomic, e.g., by pre- and postsynaptic modulation of glutamatergic transmission, and genomic mechanisms \( 37,38 \). The well-established effects of glucocorticoids on synaptic function raise the possibility that the elevated cortisol underlies VEP plasticity impairments in BD type II. However, we found no significant association between saliva cortisol and VEP plasticity in the individuals with BD type II and their plasticity reduction remained significant after controlling group analyses for cortisol. Together, these findings indicate that impaired plasticity of the VEP in BD type II is not caused by elevated cortisol and other potential mechanisms should be addressed in future studies. In particular, BD risk genes have been linked to synaptic function and plasticity regulation \( 46-51 \), and investigations of whether and how BD risk variants affect plasticity of the VEP are warranted.

We also found significant correlations between VEP plasticity and averaged saliva cortisol and the cortisol awakening response in controls (Fig. 3). Although speculative, these positive associations could reflect the ascending part of an inverted U-shaped relationship between cortisol and synaptic plasticity. This hypothesis could be tested in future studies of VEP plasticity by including more individuals with higher stress and cortisol levels than the present work. To our knowledge, there is no other study of cortisol and plasticity of the VEP, yet two previous studies reported significant relationships between cortisol and TMS-induced motor cortex plasticity \( 44,58 \). Sale et al. \( 58 \) found that motor cortex plasticity was greater in the evening (when endogenous cortisol is lower) than in the morning and that an oral dose of hydrocortisone blocked the motor cortex plasticity. Clow et al. \( 44 \) observed a positive association between the cortisol awakening response and TMS-induced motor cortex plasticity, consistent with the results of the present study.

Based on the current understanding of mood regulation in humans \( 59,60 \), it is unlikely that impaired visual cortex synaptic plasticity is a central pathophysiological mechanism in BD type II. Two important, yet unresolved questions are therefore (1) to what extent does VEP plasticity reflect plasticity in brain regions believed to be important in mood disorders, such as prefrontal and temporal cortices and (2) are the putative cortical synaptic impairments in bipolar disorders confined to specific mood regulation-related regions or widespread? To our knowledge, no study has examined the association between synaptic plasticity in prefrontal and temporal regions and VEP plasticity. However, one recent investigation found significant association between motor cortex plasticity and VEP plasticity \( 51 \). Moreover, previous post mortem studies found evidence for synaptic impairments in bipolar disorders in several cortical regions, including prefrontal, temporal, and visual cortices \( 55,62-66 \). In addition, the significant association between depression severity and visual plasticity found in the present study supports the notion that VEP plasticity might be used as an indirect measure of synaptic impairments in mood regulation-related cortical regions. Nevertheless, more research is needed to clarify the relationship between VEP plasticity and synaptic plasticity in cortices implicated in mood regulation.

An unexpected finding of the present study was that patients had greater N1 and P1–N1 premodulation amplitudes than controls at follow-up. We examined
whether use of psychotropic drugs could underlie the premodulation amplitude increases and found, if anything, a trend toward smaller premodulation amplitudes in medicated than in unmedicated patients (see also Tables S1 and S2 for details). In contrast, there were no group differences in the premodulation amplitudes at baseline. The premodulation amplitude increase in patients at follow-up should therefore be considered cautiously and need to be confirmed by future research. Another important question is whether the larger premodulation amplitudes in patients at follow-up could be related to their VEP plasticity reduction, e.g., owing to a ceiling effect. However, the P1–N1 plasticity remained significantly reduced in patients after adjusting for premodulation amplitude. In addition, P1–N1 plasticity was also significantly reduced in patients at baseline when there were no group differences in the premodulation amplitudes. Altogether, it seems unlikely that the increased premodulation amplitude in patients underlie their impaired P1–N1 plasticity observed at follow-up.

The field of noninvasive LTP-like cortical plasticity assessment in humans is young and mainly encompasses repetitive visual or auditory stimulation-induced plasticity in sensory cortices (e.g., VEP plasticity), motor cortex plasticity induced by TMS or transcranial direct current stimulation (tDCS), and sleep slow wave activity (SWA) of the EEG. Previous studies found that TMS-induced motor cortex plasticity was decreased in MDD and that tDCS might increase motor cortex plasticity in depressed individuals. Sleep SWA is another potential EEG-based index of cortical synaptic plasticity and increased SWA during sleep has been linked to the rapid antidepressant response to ketamine treatment in MDD. Moreover, recent studies indicate that TMS and tDCS can alter SWA and that acoustic stimulation might enhance SWA during sleep. Each of these methodologies has strengths and limitations and we chose VEP plasticity in our studies because of its feasibility (e.g., no requirement of sleep or magnetic stimulation) and since detailed studies found that VEP plasticity in rodents exhibits core features of LTP.

The temporal stability of these noninvasive plasticity indices remains to be clarified and there has, to our knowledge, been no previous longitudinal study of sensory cortex LTP-like plasticity. There is also a scarcity of studies examining the test–retest reliability of other noninvasive plasticity indices and the limited TMS-induced motor cortex plasticity literature observed substantial variability. For example, Fratello et al. found low temporal stability when TMS-induced motor cortex plasticity was measured twice in healthy volunteers with a 1 week test–retest interval (ICC = 0.05). Thus, the results of the present study indicating moderate temporal stability of both the P1 and the N1 plasticity (ICC between 0.5 and 0.6) in controls with a test–retest interval of 2.2 years period are promising. These ICCs are also comparable to the reliability commonly found in functional magnetic resonance imaging studies with test–retest intervals of weeks to months (ICC usually between 0.33 and 0.66). We note, however, that the temporal stability of P1–N1 was low in the controls of the current study. This finding could be due to P1 and N1 modulation representing two independent plasticity indices. However, we found that P1 and N1 plasticity were significantly negatively correlated, which suggests that they may reflect at least partly overlapping mechanisms. We therefore speculate that the poor reliability might be related to the fact that P1–N1 includes the variability of both the P1 and the N1 component; this composite measure might thus have lower temporal stability than the individual VEP components. Thus, although the present results are promising, more work is needed to clarify the test–retest reliability of VEP plasticity in humans.

The present study comes with several limitations. First, the sample size was modest and larger studies are needed to clarify the relationships between VEP plasticity and comorbid psychiatric illnesses and illness course. Second, there were no significant differences in VEP plasticity between medicated and unmedicated patients, yet the number of unmedicated patients was small (n = 7), at follow-up. Further studies are therefore needed to fully clarify the effects of psychotropic drugs on VEP amplitudes and plasticity. Third, the present study did not include a BD type I or an MDD comparison group. More research is therefore required to assess whether the VEP plasticity impairments observed in patients of the present study are specific for BD type II or common neurobiological characteristics of mood disorders. Fourth, the precise neural mechanisms underlying VEP plasticity in humans remain to be fully clarified. However, rodent and human studies strongly suggest that plasticity of the VEP reflect cortical processes closely related to LTP. Fifth, our plasticity paradigm did not include a control stimulus to test whether VEP modulation was dependent on stimulation properties. However, frequency- and pattern-specific VEP potentiation has been demonstrated previously for the current paradigm. Sixth, we employed a limited number of electrodes. The use of high-density EEG or magnetoencephalography may increase our understanding of how modulation of cortical excitability might change neural circuitry in mood disorders. Seventh, whereas VEP plasticity was examined longitudinally, salivary cortisol was measured only at follow-up. Finally, future research could also examine whether other stress-related indices are associated with VEP plasticity, such as hair cortisol, heart rate variability, and alpha-amylase levels.

In conclusion, the present study provides additional evidence for impaired LTP-like cortical plasticity in BD
type II, suggests that impaired cortical plasticity is present in the euthymic phases of the illness and may further deteriorate during depressive episodes, and indicates that elevated cortisol does not underlie the plasticity impairment. The results also suggest a positive association between the VEP plasticity and saliva cortisol in controls, possibly reflecting an inverted U-shaped relationship between cortisol and synaptic plasticity. From a methodological perspective, converging lines of evidence indicate that synaptic dysfunction is a central pathophysiological mechanism across psychiatric illnesses and there is therefore a substantial need for techniques, which enable assessment of cortical synaptic function and plasticity in humans. The previous 24,25,28–30 and present works together suggest that VEP plasticity is an accessible method for noninvasive studies of LTP-like cortical processes, which may help elucidate the pathophysiological and clinical significance of synaptic dysfunction in psychiatric disorders.

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Conflict of interest
The authors declare that they have no conflict of interest.

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References
Figure S1. VEP plasticity of the longitudinal sample at baseline. (A) Grand average premodulation (blue) and postmodulation (red) VEP in controls (n=29). There was significant P1, N1 and P1-N1 plasticity. (B) Grand average premodulation (blue) and postmodulation (red) VEP in patients with BD type II (n=16). In contrast to controls, there was no P1, N1, or P1-N1 plasticity in patients (all p>.05). (C) VEP plasticity in patients with BD type II and controls. P1-N1 plasticity was significantly reduced in patients relative to controls. **p=.001. Error bars represent the s.e.m. Note that the grand average ERP displayed in A and B represents mean amplitude across participants for each (absolute) time-point following stimulus onset, while all statistical analyses were conducted on peak amplitudes individually determined for each participant (displayed in C). Also, two patients included in B did not display a reliable C1 component, and was hence excluded from the C1 analysis in C. Thus, the values in A and B do not strictly correspond to those in C, especially for the C1 component. VEP, visual evoked potential. BD, bipolar disorder.
Figure S2. Change in P1-N1 amplitude at the six postmodulation blocks relative to premodulation. (A) The postmodulation P1-N1 amplitudes differed significantly from the premodulation amplitude for all six postmodulation blocks (five out of six surviving Bonferroni correction) in healthy individuals. *<i>p</i> = .32, **<i>p</i> = .001, ***<i>p</i> < .001. (B) Only the P1-N1 amplitude at the first postmodulation block differed significantly from the premodulation amplitude in bipolar disorder type II. *<i>p</i> = .003.

Figure S3. VEP plasticity of the cross-sectional sample at follow-up. (A) There were no correlations between YMRS score and P1 plasticity or (B) P1-N1 plasticity in patients with bipolar disorder type II. VEP, visual evoked potential. YMRS, Young Mania Rating Scale.
Figure S2. Change in P1-N1 amplitude at the six postmodulation blocks relative to premodulation. (A) The postmodulation P1-N1 amplitudes differed significantly from the premodulation amplitude for all six postmodulation blocks (five out of six surviving Bonferroni correction) in healthy individuals. * \( p = .32 \) ** \( p = .001 \) *** \( p < .001 \) (B) Only the P1-N1 amplitude at the first postmodulation block differed significantly from the premodulation amplitude in bipolar disorder type II. * \( p = .003 \)

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SUPPLEMENTARY TABLES

Table S1. Effects of medication use on premodulation N1 amplitude in patients of the cross-sectional sample at follow-up. Only psychotropic drugs with current users of n ≥ 3 of the sample are shown.

<table>
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<tr>
<th>Medication</th>
<th>n (%)</th>
<th>Amplitude (μV (SD)) in patients not using drug (NU)</th>
<th>Amplitude (μV (SD)) in patients using drug (U)</th>
<th>Mean amplitude (μV (SD)) in controls (C)</th>
<th>Statistics</th>
<th>Post hoc test, p-value (Bonferroni corrected)</th>
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<td>All antidepressants</td>
<td>7 (24.1)</td>
<td>-11.8 (6.8)</td>
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<td>-7.9 (4.0)</td>
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<td>Any medication</td>
<td>22 (75.9)</td>
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<td>-9.9 (2.4)</td>
<td>-7.9 (4.0)</td>
<td>$F_{2,55}=3.58$</td>
<td>$p=.035$</td>
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SD, standard deviation

Table S2. Effects of medication use on premodulation P1-N1 amplitude in patients of the cross-sectional sample at follow-up. Only psychotropic drugs with current users of n ≥ 3 of the sample are shown.

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<th>Statistics</th>
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<tr>
<td>All antidepressants</td>
<td>7 (24.1)</td>
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<td>13.5 (6.6)</td>
<td>9.3 (4.7)</td>
<td>$F_{2,55}=7.15$</td>
<td>$p=.002$</td>
</tr>
<tr>
<td>SSRIs</td>
<td>3 (10.3)</td>
<td>15.4 (7.1)</td>
<td>11.4 (2.0)</td>
<td>9.3 (4.7)</td>
<td>$F_{2,55}=7.53$</td>
<td>$p=.001$</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>16 (55.2)</td>
<td>15.3 (8.1)</td>
<td>14.8 (6.0)</td>
<td>9.3 (4.7)</td>
<td>$F_{2,55}=6.77$</td>
<td>$p=.002$</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>3 (10.3)</td>
<td>15.4 (7.1)</td>
<td>12.1 (3.8)</td>
<td>9.3 (4.7)</td>
<td>$F_{2,55}=7.27$</td>
<td>$p=.002$</td>
</tr>
<tr>
<td>Any medication</td>
<td>22 (75.9)</td>
<td>16.7 (9.5)</td>
<td>14.5 (6.0)</td>
<td>9.3 (4.7)</td>
<td>$F_{2,55}=7.20$</td>
<td>$p=.002$</td>
</tr>
</tbody>
</table>

SD, standard deviation
Table S3. Effects of medication use on N1 plasticity in patients of the cross-sectional sample at follow-up. Only psychotropic drugs with current users of n ≥ 3 of the sample are shown.

<table>
<thead>
<tr>
<th>Medication</th>
<th>n (%)</th>
<th>Amplitude change (μV (SD)) in patients not using drug (NU)</th>
<th>Amplitude change (μV (SD)) in patients using drug (U)</th>
<th>Amplitude change (μV (SD)) in controls (C)</th>
<th>Statistics</th>
<th>Post hoc test, p-value (Bonferroni corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All antidepressants</td>
<td>7 (24.1)</td>
<td>-.24 (2.6)</td>
<td>.68 (2.1)</td>
<td>1.39 (2.7)</td>
<td>$F_{2,35}=2.45$</td>
<td>$p=.096$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td>SSRIs</td>
<td>3 (10.3)</td>
<td>-.34 (2.5)</td>
<td>2.82 (0.5)</td>
<td>1.39 (2.7)</td>
<td>$F_{2,35}=4.37$</td>
<td>$p=.017$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NU&lt;C,.041</td>
<td></td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>16 (55.2)</td>
<td>.56 (2.3)</td>
<td>-.48 (2.7)</td>
<td>1.39 (2.7)</td>
<td>$F_{2,35}=2.72$</td>
<td>$p=.075$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td>Quetiapine</td>
<td>3 (10.3)</td>
<td>-.14 (2.6)</td>
<td>1.01 (1.1)</td>
<td>1.39 (2.7)</td>
<td>$F_{2,35}=2.37$</td>
<td>$p=.103$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td>Any medication</td>
<td>22 (75.9)</td>
<td>1.00 (2.8)</td>
<td>-.34 (2.4)</td>
<td>1.39 (2.7)</td>
<td>$F_{2,35}=2.84$</td>
<td>$p=.067$</td>
</tr>
</tbody>
</table>

SD, standard deviation

Table S4. Effects of medication use on P1-N1 plasticity in patients of the cross-sectional sample at follow-up. Only psychotropic drugs with current users of n ≥ 3 of the sample are shown.

<table>
<thead>
<tr>
<th>Medication</th>
<th>n (%)</th>
<th>Amplitude change (μV (SD)) in patients not using drug (NU)</th>
<th>Amplitude change (μV (SD)) in patients using drug (U)</th>
<th>Amplitude change (μV (SD)) in controls (C)</th>
<th>Statistics</th>
<th>Post hoc test, p-value (Bonferroni corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All antidepressants</td>
<td>7 (24.1)</td>
<td>.64 (2.5)</td>
<td>.26 (3.7)</td>
<td>2.39 (1.9)</td>
<td>$F_{2,35}=4.38$</td>
<td>$p=.017$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NU&lt;C,.037</td>
<td></td>
</tr>
<tr>
<td>SSRIs</td>
<td>3 (10.3)</td>
<td>.47 (2.9)</td>
<td>1.30 (0.9)</td>
<td>2.39 (1.9)</td>
<td>$F_{2,35}=4.49$</td>
<td>$p=.016$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NU&lt;C,.012</td>
<td></td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>16 (55.2)</td>
<td>.93 (2.0)</td>
<td>.24 (3.3)</td>
<td>2.39 (1.9)</td>
<td>$F_{2,35}=4.66$</td>
<td>$p=.014$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>U&lt;C,.016</td>
<td></td>
</tr>
<tr>
<td>Quetiapine</td>
<td>3 (10.3)</td>
<td>.51 (2.8)</td>
<td>.87 (2.2)</td>
<td>2.39 (1.9)</td>
<td>$F_{2,35}=4.34$</td>
<td>$p=.018$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NU&lt;C,.016</td>
<td></td>
</tr>
<tr>
<td>Any medication</td>
<td>22 (75.9)</td>
<td>.51 (1.5)</td>
<td>.57 (3.1)</td>
<td>2.39 (1.9)</td>
<td>$F_{2,35}=4.30$</td>
<td>$p=.018$</td>
</tr>
</tbody>
</table>

SD, standard deviation
Table S5. Effects of adding a psychotropic drug on VEP plasticity changes in patients from baseline to follow-up.

<table>
<thead>
<tr>
<th>VEP component</th>
<th>Amplitude change from baseline to follow-up (μV (SD)) in patients who added medication (n=5)</th>
<th>Amplitude change from baseline to follow-up (μV (SD)) in other patients (n=11)</th>
<th>Amplitude change from baseline to follow-up (μV (SD)) in controls (n=29)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>-1.82 (3.7)</td>
<td>.83 (1.7)</td>
<td>-.97 (3.2)</td>
<td>$F_{2,42} = .78$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$p = .465$</td>
</tr>
<tr>
<td>P1</td>
<td>-.54 (5.7)</td>
<td>1.10 (3.1)</td>
<td>.32 (2.4)</td>
<td>$F_{2,42} = .43$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$p = .657$</td>
</tr>
<tr>
<td>N1</td>
<td>-1.47 (4.9)</td>
<td>1.61 (2.5)</td>
<td>.30 (2.4)</td>
<td>$F_{2,42} = 1.98$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$p = .151$</td>
</tr>
<tr>
<td>P1N1</td>
<td>.93 (2.4)</td>
<td>-.51 (1.3)</td>
<td>.02 (2.8)</td>
<td>$F_{2,42} = .70$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$p = .503$</td>
</tr>
</tbody>
</table>

SD, standard deviation
**TITLE:** Mood episodes are associated with increased cortical thinning: a longitudinal study of bipolar disorder type II

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**SHORT TITLE:** Cortical Thinning in Bipolar Disorder II

**NUMBER OF WORDS IN ABSTRACT AND ARTICLE BODY:** 249 and 4996

**NUMBER OF FIGURES, TABLES AND SUPPLEMENTAL INFORMATION:** 3 figures, 4 tables, and 1 supporting information
Abstract

Objectives: Previous studies found evidence for thinner frontotemporal cortices in bipolar disorder (BD), yet whether this represents a stable disease trait or an effect of mood episodes remains unknown. Here we assessed the reproducibility of thinner frontotemporal cortices in BD type II, compared longitudinal changes in cortical thickness between individuals with BD type II and healthy controls (HCs), and examined the effect of mood episodes on cortical thickness change.

Methods: Thirty-three HCs and 29 individuals with BD type II underwent 3T magnetic resonance imaging at baseline, as published previously, and 2.4 years later, at follow-up. Cross-sectional and longitudinal analyses of cortical thickness were performed using Freesurfer, and relationships with mood episodes from baseline to follow-up were assessed.

Results: Individuals with BD type II had thinner left and right prefrontal and left temporal cortex clusters at follow-up (all corrected $P<.001$), consistent with baseline results. Both groups showed widespread longitudinal cortical thinning, and patients had increased thinning in a left temporal cortex cluster compared to HCs (corrected $P<.001$). Patients with more (>2) depressive episodes between baseline and follow-up had greater left temporal cortical thinning than patients with fewer depressive episodes (corrected $P<.05$). In addition, patients with more depressive episodes had greater thinning in bilateral ventromedial prefrontal clusters relative to HCs (uncorrected $P<.05$), yet these results did not survive correction for multiple comparisons.

Conclusions: Together, these findings support reduced frontotemporal cortical thickness in BD type II and provide the first preliminary evidence for an association between depressive episodes and increased cortical thinning.

KEY WORDS: bipolar disorder, depressive episodes, cortical thickness, cortical thinning, longitudinal study, progressive changes
1 | INTRODUCTION

Bipolar disorder (BD) is a chronic psychiatric illness characterized by episodic disturbances in mood and activity levels, and affects 2-3% of the population worldwide.\textsuperscript{1} BD type I is defined by at least one manic episode, while BD type II is characterized by alternating episodes of hypomania and depression. The clinical significance of both subtypes are comparable in terms of illness burden, role impairments, and suicide attempts.\textsuperscript{1} Depressive symptoms dominate the longitudinal course of BD, and account for most of patients' lifetime disability and suffering.\textsuperscript{2} Individuals with BD can experience a progressive illness course with shortening of inter-episode intervals and impaired treatment response.\textsuperscript{3} Although the neural underpinnings of illness progression remain poorly understood, it has been suggested that mood episodes can cause lasting neurobiological alterations.\textsuperscript{4}

Recent neuroimaging studies found thinner prefrontal and temporal cortices in BD type I and II.\textsuperscript{5,6} These cortical regions are believed to play central roles in processing and regulation of emotions.\textsuperscript{7-9} One recent study found a positive correlation between cortical thickness in medial prefrontal regions and executive function in individuals with BD type II, but not in BD type I,\textsuperscript{10} yet there is still a scarcity of neuroimaging studies in BD type II. There are also few longitudinal cortical thickness studies in BD, and whether frontotemporal cortical thinning is a stable trait predisposing to illness development or an effect of mood episodes remains to be clarified. Only one longitudinal study has examined the effects of mood episodes on cortical thinning in BD and found increased frontal thinning in patients who experienced manic episodes during follow-up.\textsuperscript{11} Two cross-sectional studies of cortical thickness in BD type I and II suggested thinner prefrontal cortices in individuals with higher lifetime number of depressive episodes.\textsuperscript{12,13} One cross-sectional study of major depressive disorder (MDD),\textsuperscript{14} but not others,\textsuperscript{13,15,16} observed a negative association between number of previous depressive episodes and medial prefrontal and temporal cortical thickness.
Furthermore, a recent longitudinal study found that individuals with depressive symptoms at baseline had increased thinning in left frontal and bilateral temporal cortices and temporoparietal junctions compared to a control group.\textsuperscript{17}

Here, we analyzed structural magnetic resonance imaging (MRI) data from individuals with BD type II and healthy controls (HCs) at baseline and 2.4 years later, at follow-up. At baseline, we found thinner bilateral prefrontal and temporal cortices in BD type II, but no group differences in surface area, as reported previously.\textsuperscript{18} The aims of the present longitudinal study were to assess the reproducibility of thinner frontotemporal cortices in BD type II at follow-up, to compare changes in cortical thickness from baseline to follow-up between individuals with BD type II and HCs, and to examine whether changes in cortical thickness were associated with mood episodes in the patients.
Furthermore, a recent longitudinal study found that individuals with depressive symptoms at baseline had increased thinning in left frontal and bilateral temporal cortices and temporaparietal junctions compared to a control group.17 Here, we analyzed structural magnetic resonance imaging (MRI) data from individuals with BD type II and healthy controls (HCs) at baseline and 2.4 years later, at follow-up. At baseline, we found thinner bilateral prefrontal and temporal cortices in BD type II, but no group differences in surface area, as reported previously.18 The aims of the present longitudinal study were to assess the reproducibility of thinner frontotemporal cortices in BD type II at follow-up, to compare changes in cortical thickness from baseline to follow-up between individuals with BD type II and HCs, and to examine whether changes in cortical thickness were associated with mood episodes in the patients.

2 | MATERIALS AND METHODS

Study Design and Participants

Seventy-eight subjects underwent MRI at baseline, as previously reported.18 Sixty-two participants (33HCs and 29 individuals with BD type II) were reexamined on average 2.4 years later, and were included in longitudinal and cross-sectional analyses, at follow-up. Reasons for not participating in the follow-up study were: moved out of the area (1 patient, 5 HCs), exclusion at follow-up due to a newly diagnosed premalign condition (1 HC), did not attend or withdrew consent (6 patients, 2 HCs), claustrophobia (2 patients), and pregnancy (1 HC). In addition, seven new patients and two new HCs underwent MRI at follow-up; thus, the cross-sectional sample at follow-up comprised 71 participants.

Patients were recruited from psychiatric outpatient clinics in the Oslo area, and clinical examinations at baseline and follow-up were carried out by three senior psychiatrists (i.e., authors EB, BB, and UFM) at a university department specializing in evaluation and treatment of mood disorders. Axis I diagnoses and psychiatric comorbidities were determined at both time points using the Mini-International Neuropsychiatric Interview (MINI), Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria version 5.0.19 Demographic and supplementary information was obtained through a semi-structured interview using the Stanley Foundation Network Entry Questionnaire.20 Number of mood episodes from baseline to follow-up was assessed during this interview. Alcohol and drug abuse were assessed with the Alcohol Use Scale and the Drug Use Scale,21 respectively. Mood state at the time of MRI scanning was determined by the Montgomery–Asberg Depression Rating Scale (MADRS)22 and the Young Mania Rating Scale (YMRS).23

Controls were recruited through local advertising and had no previous or current psychiatric illness. They underwent a full clinical examination similar to that of the patients. The exclusion criteria for all subjects were: age <18 or >50 years, previous head injury with
loss of consciousness for >1 min, history of neurological or other severe chronic somatic disorder, and pregnancy. The Regional Ethical Committee of South-Eastern Norway approved the study, and all subjects provided written informed consent to participate after receiving written and oral information about the study.

**MRI Acquisition and Analysis**

Imaging at baseline and follow-up was performed on the same 3T Philips Achieva Scanner (Philips Healthcare, Eindhoven, the Netherlands) using an eight-channel SENSE head coil. Structural brain images were acquired using a T1-weighted three-dimensional turbo field echo (TFE) sequence [repetition time (TR)/echo time (TE)=8.4msec/2.3msec, field of view (FOV)=256mm × 256mm × 220mm, 1mm isotropic resolution, TA = 7min 40sec).

All data sets were processed blindly and analyzed at the Neuroimaging Analysis Lab at the Norwegian Centre of Mental Disorders Research at Oslo University Hospital. Freesurfer software version 5.3 was used to automatically reconstruct cortical surfaces from T1-weighted MRI-images, details regarding the surface-based analysis are provided elsewhere.\(^{24-26}\) Briefly, processing steps include removal of non-brain tissue,\(^{27}\) automated Talairach transformation, and intensity correction. Information about intensity and continuity from the three-dimensional volume is used in segmentation and deformation procedures to reconstruct a gray/white matter boundary across the brain.\(^{24}\) Cortical surfaces are then inflated, registered to a spherical atlas, and gyral and sulcal regions are identified automatically.\(^{27}\) Reconstructed data sets were visually inspected for accuracy at several points along the processing pipeline, and segmentation errors were manually corrected, re-processed, and re-inspected. Cortical thickness maps were obtained for each participant by calculating the distance between the gray and white matter surfaces at each vertex, and compared across groups.
For longitudinal analyses the implemented longitudinal stream in Freesurfer was used to prepare surfaces for comparisons of baseline and follow-up examinations. This procedure processes longitudinal data with common information from a template created for each subject, thereby reducing measurement noise and increasing precision. Temporal data within each subject was then reduced to rate of change maps (mm/year = thick2-thick1/time2-time1) using the long_mris_slopes function in Freesurfer’s longitudinal two-stage model, and these were compared across groups. Before statistical analysis, cortical thickness maps processed for cross-sectional and longitudinal analyses were smoothed with a full width of half maximum Gaussian kernel of 20mm, as in the previously published baseline analyses.

**Statistical Analyses**

**Cross-sectional and longitudinal analyses of cortical thickness**

Cross-sectional and longitudinal surface-based analyses were performed using vertex-wise general linear models (GLMs). To reduce the probability of type I errors, all surface-based analyses were corrected for multiple comparisons using cluster size inference by means of Z Monte Carlo simulations, as implemented in Freesurfer. Here, clusters were tested against an empirical null distribution of maximum cluster size built using synthesized Z distributed data across 10,000 permutations, yielding clusters fully corrected for multiple comparisons across the surface. The initial cluster-forming threshold employed was $P<.05$. Longitudinal analyses of thickness change (mm/year) were performed using the mri_glmfit function in Freesurfer’s longitudinal two-stage model. Main group effects on cortical thickness and thickness change were tested by contrasting subjects with BD type II and HCs, while controlling for age and sex. The longitudinal patient sample was then split in two groups based on the median number of depressive and hypomanic episodes during the follow-up period (few vs. many episodes), as number of mood episodes was assessed retrospectively, and therefore prone to recall bias.
Further longitudinal surface-based analyses were conducted pairwise contrasting the three groups: HCs vs. patients with few mood episodes, HCs vs. patients with many mood episodes, and patients with few vs. many mood episodes. For the sake of completeness, we also examined the effect of absolute number of mood episodes on cortical thinning in the patient group, and the results are shown in Supporting Figure S6. One patient was excluded from these analyses, as number of mood episodes was missing.

Analyses of demography and effects of clinical variables

Additional statistical analyses were performed using SPSS, version 24.0 for Windows (SPSS, Chicago, IL, USA). A two-tailed $P$ value of <.05 was considered statistically significant. To test for differences in demographic and clinical variables between patients and HCs, Student's $t$-test, the $\chi^2$ test, and the Fisher’s exact test were performed. To examine the relationship between cortical thickness changes in BD type II and number of mood episodes between baseline and follow-up, mean rates of thickness change (mm/year) for each participant in clusters showing significant between-group differences were computed, and these values were also used to calculate effect sizes (Cohen’s $d$). GLMs, with Bonferroni-corrected post-hoc tests, were then employed to test the effect of mood episodes (many vs. few vs. HCs) on cluster mean values. Effects of depressive episodes, when present, were then corrected for hypomanic episodes, and vice versa. In exploratory analyses, we assessed the effects of illness duration (years since first mood episode), medication (use vs. no use of antidepressants, mood stabilizers, antipsychotic medications, and any psychotropic medication; see Table 1 and 2 for details), and family history of BD (patients with vs. patients without first-degree relatives with BD), on cluster mean values in the patient group, while controlling for age and sex. Analyses were also run while excluding subjects with alcohol abuse, cigarette smoking, and non-
Further longitudinal surface-based analyses were conducted pairwise contrasting the three groups: HCs vs. patients with few mood episodes, HCs vs. patients with many mood episodes, and patients with few vs. many mood episodes. For the sake of completeness, we also examined the effect of absolute number of mood episodes on cortical thinning in the patient group, and the results are shown in Supporting Figure S6. One patient was excluded from these analyses, as number of mood episodes was missing.

Analyses of demography and effects of clinical variables

Additional statistical analyses were performed using SPSS, version 24.0 for Windows (SPSS, Chicago, IL, USA). A two-tailed P value of <.05 was considered statistically significant. To test for differences in demographic and clinical variables between patients and HCs, Student's t-test, the χ² test, and the Fisher's exact test were performed. To examine the relationship between cortical thickness changes in BD type II and number of mood episodes between baseline and follow-up, mean rates of thickness change (mm/year) for each participant in clusters showing significant between-group differences were computed, and these values were also used to calculate effect sizes (Cohen's d). GLMs, with Bonferroni-corrected post-hoc tests, were then employed to test the effect of mood episodes (many vs. few vs. HCs) on cluster mean values. Effects of depressive episodes, when present, were then corrected for hypomanic episodes, and vice versa. In exploratory analyses, we assessed the effects of illness duration (years since first mood episode), medication (use vs. no use of antidepressants, mood stabilizers, antipsychotic medications, and any psychotropic medication; see Table 1 and 2 for details), and family history of BD (patients with vs. patients without first-degree relatives with BD), on cluster mean values in the patient group, while controlling for age and sex. Analyses were also run while excluding subjects with alcohol abuse, cigarette smoking, and non-euthymic patients. In order to test for possible outlier effects we reran the analyses after removing subjects with studentized residuals of >|2.0|.
3 | RESULTS

Characteristics and Analyses of the Cross-Sectional Sample at Follow-up

Sample characteristics

Demographic and clinical characteristics of the study participants (n=71) at follow-up are shown in Table 1. There were no significant differences in age or sex distributions between patients (n=36, mean [SD] age, 36.4 [7.5] years; 26 women) and HCs (n=35, 35.0 [9.4] years; 19 women). Patients had lower educational level and higher body mass index (BMI) than HCs. No subjects had substance abuse, while three patients abused alcohol. Twenty-six of the patients were medicated, and mood stabilizers (n=21) and antidepressants (n=10) were the most frequently used medications. Panic disorder and social phobia were common comorbid diagnoses. Illness duration was 18.0±6.8 years. Twenty patients were euthymic (MADRS score <11 and YMRS score <8), three patients were hypomanic (YMRS score 8), nine patients were mildly depressed (MADRS score range 12-18), and four patients were moderately depressed (MADRS score range 21-27) at the time of MRI.

Cortical thickness

Patients versus HCs

Patients had significantly thinner cortices at follow-up relative to HCs in 1) a left prefrontal cluster comprising dorsolateral, ventromedial, and dorsomedial prefrontal cortices, 2) a right prefrontal cluster comprising dorsolateral, ventromedial, and dorsomedial prefrontal cortices and perigenual anterior cingulate cortex, and 3) a left anterior temporal cluster comprising the superior, middle and inferior temporal gyri (Figure 1A and Table 3, all corrected \( P=0.0001 \); see Supporting Figure S1C for uncorrected results). The group differences in mean cortical thickness in the left, right prefrontal and left temporal clusters were 4.6% (mean±SD, 2.22±0.12mm in patients vs. 2.32±0.13mm in HCs, Cohen’s \( d=0.87 \)), 5.0% (2.18±0.12mm vs. 2.30±0.13mm, Cohen’s \( d=0.91 \)), and 5.4% (2.64±0.15mm vs. 2.79±0.15mm, Cohen’s \( d=0.99 \)), respectively. No region showed significantly thicker cortex in patients compared to controls. Follow-up analyses

Group differences remained significant after controlling for educational level and BMI (all \( P≤0.006 \)). There were no thickness differences in these clusters between patients who used and patients who did not use psychotropic medication, antidepressants, mood stabilizers, and antipsychotics (all \( P>0.05 \)). Furthermore, group differences remained significant after excluding the three patients with alcohol abuse and after excluding six patients and four controls who were smokers (all \( P≤0.004 \)). Group differences remained significant when only euthymic patients (n=20) were included in the analyses (all \( P<0.003 \)). Similar group differences were found when using a cluster-forming threshold of <.01 (Supporting Figure S1D).
Characteristics and Analyses of the Cross-Sectional Sample at Follow-up

Sample characteristics

Demographic and clinical characteristics of the study participants (n=71) at follow-up are shown in Table 1. There were no significant differences in age or sex distributions between patients (n=36, mean [SD] age, 36.4 [7.5] years; 26 women) and HCs (n=35, 35.0 [9.4] years; 19 women). Patients had lower educational level and higher body mass index (BMI) than HCs. No subjects had substance abuse, while three patients abused alcohol. Twenty-six of the patients were medicated, and mood stabilizers (n=21) and antidepressants (n=10) were the most frequently used medications. Panic disorder and social phobia were common comorbid diagnoses. Illness duration was 18.0±6.8 years. Twenty patients were euthymic (MADRS score <11 and YMRS score <8), three patients were hypomanic (YMRS score 8), nine patients were mildly depressed (MADRS score range 12-18), and four patients were moderately depressed (MADRS score range 21-27) at the time of MRI.

Follow-up analyses

Group differences remained significant after controlling for educational level and BMI (all $P \leq .006$). There were no thickness differences in these clusters between patients who used and patients who did not use psychotropic medication, antidepressants, mood stabilizers, and antipsychotics (all $P > .05$). Furthermore, group differences remained significant after excluding the three patients with alcohol abuse and after excluding six patients and four controls who were smokers (all $P \leq .004$). Group differences remained significant when only euthymic patients (n=20) were included in the analyses (all $P < .003$). Similar group differences were found when using a cluster-forming threshold of <.01 (Supporting Figure S1D).

Characteristics and Analyses of the Longitudinal Sample

Sample characteristics

Demographic and clinical data for the longitudinal sample (n=62) are shown in Table 2. There were no significant differences in age or sex between patients (n=29, 33.3 [6.8] years; 20 women) and HCs (n=33, 32.5 [9.4] years; 18 women). There was a small, but significant difference in the interval between scans (2.3 years in patients vs. 2.4 years in HCs). This was accounted for in the analyses, which were performed on the annual change (mm/year) of cortical thickness. Patients had higher BMI than controls, and panic disorder and social phobia were frequent comorbid conditions. Twenty-six patients were using psychotropic drugs at both time points. Eighteen patients were euthymic (MADRS score <11 and YMRS score <8), one patient was hypomanic (YMRS score 8), seven patients were mildly depressed
(MADRS score 12-18), and three patients were moderately depressed (MADRS score 21-27) at the time of MRI. No subjects had substance abuse, while two patients abused alcohol. Illness duration was 17.5±6.8 years. Median number of depressive episodes between baseline and follow-up was two. Fifteen patients had 0-2 depressive episodes, while 13 patients had 3-10 episodes. Median number of hypomanic was four; twelve patients had 0-3 hypomanic episodes, while 16 patients had 4-20 episodes. There was a significant positive correlation between number of hypomanic and depressive episodes during the follow-up period ($r=.43$, $P=.022$). There were no significant differences in age, sex, scan interval, or clinical variables between patients with few and many depressive or hypomanic episodes (see Supporting Table S1 for details).

Cortical thickness change

Patients versus HCs

Patients and HCs showed widespread cortical thinning from baseline to follow-up; no regions showed significant increase in cortical thickness (Figure 1B and C, see also Supporting Figure S2C and D for maps showing rate of change). There were no significant sex differences in cortical thinning in the total sample, or within the two groups (Supporting Figure S5). Patients had significantly greater thinning than HCs in a left temporal cluster, mainly involving the middle and inferior temporal gyri, superior temporal sulcus, and the fusiform gyrus (Figure 1D and Table 4, corrected $P=.0001$; see Supporting Figure S2A for uncorrected results). The mean rates of thickness change within this cluster were $-.025±.04$mm/year for patients and $.002±.03$mm/year for HCs (Cohen’s $d=-.79$).
Follow-up analyses

The group difference remained significant after controlling for BMI ($P=.007$). Group differences remained significant after removing subjects with studentized residuals of $>|2.0|$ (two patients and one HC, $P=.003$), after excluding two patients with alcohol abuse ($P=.005$) and after excluding six patients and four HCs who were smokers ($P=.001$). Moreover, the group difference remained significant when only including currently euthymic patients ($n=18$) in the analysis ($P=.002$). Furthermore, there was no significant effect of illness duration, family history of BD, current psychotropic medication use, antidepressant use, use of mood stabilizers, or use of antipsychotics on thinning within the left temporal cluster (all $P>.05$). Similar group difference was found when using a cluster-forming threshold of $<.01$ (Supporting Figure S2B).

Effect of mood episodes on left temporal cortical thinning

Effects of depressive episodes on cortical thinning

There was a significant effect of depressive episodes (patients with many episodes ($>2$) vs. patients with few ($\leq2$) vs. HCs) on mean rate of thinning within the left temporal difference cluster ($P=.001$), and this effect remained significant after adjusting for hypomanic episodes (many ($\geq4$) vs. few ($<4$) vs. HCs, $P=.023$). Post hoc analyses showed that mean thinning rate was significantly greater in patients with many depressive episodes relative to patients with few episodes (Bonferroni-corrected $P=.039$) and HCs (Bonferroni-corrected $P=.001$; Figure 1E); no significant difference was found between patients with few depressive episodes and HCs ($P=.855$).
Effects of hypomanic episodes on cortical thinning

There was also a significant effect of hypomanic episodes (many vs. few vs. HCs) on mean rate of cortical thickness change in the left temporal difference cluster \((P=.014)\), and post hoc analysis showed that patients with few hypomanic episodes had greater mean thinning rate compared to controls (Bonferroni-corrected \(P=.039\)), however this result was no longer significant after adjusting for depressive episodes (many vs. few vs. HCs, \(P=.603\)). There were no significant correlations between mean thinning rate in the left temporal cluster and absolute number of hypomanic, depressive or total number of mood episodes between baseline and follow-up. There was no sign of multicollinearity and no significant interaction effect of depressive and hypomanic episodes (absolute number and dichotomized) on mean rate of thinning in the left temporal difference cluster.

Whole-brain analysis of thickness changes and mood episodes

Based on the finding of significant effect of many depressive episodes on thickness change in the left temporal difference cluster, we then ran a longitudinal whole-brain thickness analysis pairwise contrasting the three groups (many depressive episodes vs. few vs. HCs; Figure 2).

Many depressive episodes versus few depressive episodes

We found significantly greater cortical thinning in an overlapping left temporal region in patients with many depressive episodes relative to patients with few episodes (corrected \(P=.0013\), Figure 2A; \(-.048\pm.01\) mm/year vs. \(<.001\pm.01\) mm/year, Cohen’s \(d=−1.17\)), which remained significant after adjusting for hypomanic episodes (many vs. few, \(P=.01\)). Details regarding the cluster are shown in Table 4. Furthermore, patients with many depressive episodes had greater thinning in a right prefrontal cluster relative to patients with few depressive episodes (Supporting Figure S3D; \(-.027\pm.02\) mm/year vs. \(.036\pm.01\) mm/year, \(P\)
There was also a significant effect of hypomanic episodes (many vs. few vs. HCs) on mean rate of cortical thickness change in the left temporal difference cluster ($P = .014$), and post hoc analysis showed that patients with few hypomanic episodes had greater mean thinning rate compared to controls (Bonferroni-corrected $P = .039$), however this result was no longer significant after adjusting for depressive episodes (many vs. few vs. HCs, $P = .603$). There were no significant correlations between mean thinning rate in the left temporal cluster and absolute number of hypomanic, depressive or total number of mood episodes between baseline and follow-up. There was no sign of multicollinearity and no significant interaction effect of depressive and hypomanic episodes (absolute number and dichotomized) on mean rate of thinning in the left temporal difference cluster.

**Whole-brain analysis of thickness changes and mood episodes**

Based on the finding of significant effect of many depressive episodes on thickness change in the left temporal difference cluster, we then ran a longitudinal whole-brain thickness analysis pairwise contrasting the three groups (many depressive episodes vs. few vs. HCs; Figure 2).

**Patients with many depressive episodes versus HCs**

Patients with many depressive episodes had increased thinning in a left temporal region relative to patients with few episodes (corrected $P = .001$, Figure 2A; -.044±0.01mm/year vs. -.005±0.01mm/year, Cohen’s $d = -1.17$), which remained significant after adjusting for hypomanic episodes (many vs. few, $P = .01$). Details regarding the cluster are shown in Table 4. Furthermore, patients with many depressive episodes had greater thinning in a right prefrontal cluster relative to patients with few depressive episodes (Supporting Figure S3D; -.027±0.02mm/year vs. .036±0.01mm/year, $P = .018$, Cohen’s $d = -0.86$), however, this result did not survive correction for multiple comparisons.

**Patients with few depressive episodes versus HCs**

There were no significant differences in cortical thinning between patients with few depressive episodes and HCs.

**Effects of hypomanic episodes**

In exploratory analyses of potential effects of hypomanic episodes on cortical thinning, we ran longitudinal whole-brain analyses pairwise contrasting patients with many hypomanic episodes between baseline and follow-up, patients with few hypomanic episodes, and HCs (Figure 3).
Few hypomanic episodes versus many hypomanic episodes

The analyses indicated that patients with few hypomanic episodes had increased rate of thinning in bilateral temporal cortex clusters compared to patients with many hypomanic episodes (Figure 3A; right cluster: corrected $P=.0001$, $-.048\pm0.01\text{mm/year}$ vs. $-.008\pm0.01\text{mm/year}$, Cohen’s $d=−.94$; and left cluster: corrected $P=.0307$, $-.048\pm0.01\text{mm/year}$ vs. $0.001\pm0.02\text{mm/year}$, Cohen’s $d=−.89$), and these findings remained significant after adjustment for depressive episodes (many vs. few, $P=.048$ and $P=.017$, respectively).

Patients with few hypomanic episodes versus HCs

These exploratory analyses also indicated that patients with few hypomanic episodes had increased rate of thinning in bilateral temporal cortex clusters (Figure 3B; right cluster: corrected $P=.0001$, $-.048\pm0.01\text{mm/year}$ vs. $-.010\pm0.01\text{mm/year}$, Cohen’s $d=−.99$; and left cluster: corrected $P=.0001$, $-.037\pm0.01\text{mm/year}$ vs. $-.009\pm0.005\text{mm/year}$, Cohen’s $d=−.70$) and in a right ventromedial prefrontal cortex cluster (corrected $P=.0023$, $-.053\pm0.02\text{mm/year}$ vs. $-.007\pm0.01\text{mm/year}$, Cohen’s $d=−.98$) compared to HCs. Details regarding the clusters are shown in Supporting Table S2.

Patients with many hypomanic episodes versus HCs

There were no significant differences between patients with many hypomanic episodes and HCs.

Effects of total number of mood episodes

Whole-brain analyses of relationships between cortical thickness changes and absolute number of depressive, hypomanic and total number of mood episodes were also run and there
were no significant associations after corrections for multiple analyses (See Supporting Figure S6 for uncorrected results).

Cortical thickness at baseline for the longitudinal subsample

We also ran cross-sectional thickness analyses for the longitudinal subsample at baseline (n=62) and found thinner prefrontal cortices bilaterally in patients (see Supporting Figure 1A; all corrected $P<.05$), consistent with the results of the whole baseline sample (n=78), as published previously.\textsuperscript{18} We then examined whether cortical thickness within these prefrontal regions could predict number of mood episodes in the patients between baseline and follow-up, but found no significant associations (all $P>.05$).
DISCUSSION

The present longitudinal study of cortical thickness in BD type II had three main findings. First, consistent with the baseline results,\(^{18}\) we found thinner cortices in frontotemporal regions in BD type II compared to HCs at follow-up, involving bilateral dorsolateral, ventromedial, and dorsomedial prefrontal cortices, right perigenual anterior cingulate cortex, and left temporal cortices. Second, whereas both groups exhibited widespread cortical thinning over 2.4 years, patients had greater thinning over time in left posterior and inferior temporal cortices. Third, individuals with higher number of depressive episodes between baseline and follow-up had greater left temporal cortical thinning over time than patients with fewer depressive episodes and HCs. In addition, patients with more depressive episodes had increased thinning of bilateral ventromedial prefrontal cortices from baseline to follow-up, yet these effects did not remain significant after correcting for analyses across the cortex. Together, these findings provide additional support for bilateral frontotemporal cortical thinning in BD type II and, although preliminary, the first longitudinal evidence linking depressive episodes to increased cortical thinning in mood disorders.

The present study suggests that depressive episodes are associated with increased thinning of temporal and ventromedial prefrontal cortices. These finding are consistent with some,\(^{12-14}\) but not all,\(^{15,29,30}\) previous cross-sectional studies of cortical thickness in BD and MDD. In addition, one study of individuals with treatment-resistant depression found that non-remitters had greater frontal cortical thinning than remitters.\(^{31}\) Furthermore, longitudinal voxel- and tensor-based morphometric studies support an association between depressive episodes and frontal and temporal gray matter (GM) loss in BD\(^{32,33}\) and MDD.\(^{34}\) Interestingly, some previous studies found an association between depressive episodes and GM loss and cortical thinning in left inferior temporal regions overlapping with the regions found in this study.\(^{14,32,33}\) Moreover, thinner left temporal cortices is one of the most consistent findings
The present longitudinal study of cortical thickness in BD type II had three main findings. First, consistent with the baseline results, we found thinner cortices in frontotemporal regions in BD type II compared to HCs at follow-up, involving bilateral dorsolateral, ventromedial, and dorsomedial prefrontal cortices, right perigenual anterior cingulate cortex, and left temporal cortices. Second, whereas both groups exhibited widespread cortical thinning over 2.4 years, patients had greater thinning over time in left posterior and inferior temporal cortices. Third, individuals with higher number of depressive episodes between baseline and follow-up had greater left temporal cortical thinning over time than patients with fewer depressive episodes and HCs. In addition, patients with more depressive episodes had increased thinning of bilateral ventromedial prefrontal cortices from baseline to follow-up, yet these effects did not remain significant after correcting for analyses across the cortex. Together, these findings provide additional support for bilateral frontotemporal cortical thinning in BD type II and, although preliminary, the first longitudinal evidence linking depressive episodes to increased cortical thinning in mood disorders.

The present study suggests that depressive episodes are associated with increased thinning of temporal and ventromedial prefrontal cortices. These finding are consistent with some, but not all, previous cross-sectional studies of cortical thickness in BD and MDD. In addition, one study of individuals with treatment-resistant depression found that non-remitters had greater frontal cortical thinning than remitters. Furthermore, longitudinal voxel- and tensor-based morphometric studies support an association between depressive episodes and frontal and temporal gray matter (GM) loss in BD and MDD. Interestingly, some previous studies found an association between depressive episodes and GM loss and cortical thinning in left inferior temporal regions overlapping with the regions found in this study. Moreover, thinner left temporal cortices is one of the most consistent findings across cortical thickness studies in BD. Although the mechanisms underlying the potential lateralization effects of BD remain unknown, genes involved in handedness and language lateralization were associated with depression and BD in a recent study. Importantly, however, uncorrected results of our analyses also indicated an association between depressive episodes and right temporal cortical thinning (Supporting Figure 2A and E) and the laterality of our results should therefore be interpreted with caution.

The precise mechanisms underlying the association between depressive episodes and increased cortical thinning, as indicated by the present study, remain to be clarified. However, depressive episodes can cause severe and long-lasting psychological stress, glucocorticoid increases, and hypothalamic-pituitary-adrenal axis dysregulation. A recent longitudinal study found that subjects with depressive symptoms at baseline had increased cortical thinning in left frontal and bilateral superior temporal and supramarginal regions compared to controls, that thinning rate in these regions correlated with cortisol levels, and that cortisol levels were associated with thinning in widespread cortical regions in a mixed sample of depressed and non-depressed individuals. In addition, BD is associated with increased levels of pro-inflammatory cytokines, and reduced levels of protective neurotrophic factors are found at later disease stages. Together, these effects may result in loss of cortical GM. In support of this notion, previous studies found loss of synapses and altered expression of genes involved in synapse morphology and formation in prefrontal cortices of depressed patients. Moreover, rodent studies reported reduced dendrite branching and synapse formation after chronic stress.

There are three previous longitudinal studies of cortical thickness in BD. The first longitudinal study found a tendency towards thinning of dorsolateral prefrontal and inferior frontal cortices in patients with BD type I who had at least one manic episode during a follow-up period of six years, and a significant decrease in cortical thickness in these regions,
compared to those with no manic episode. The second study examined change in subcortical and cortical structures over one year in patients with first-episode psychosis and HCs, and found no significant group differences; however, only a subgroup of the patients had BD. The third study assessed cortical structural changes over 7.5 years in twins who were either concordant or discordant for BD, and found no significant group differences. Further, genetic liability to BD, number of hospitalizations, and lifetime experiences of psychotic symptoms were not significantly associated with cortical thickness changes; the association with mood episodes was not examined.

We observed thinner bilateral frontotemporal cortices in BD type II at follow-up, consistent with the baseline examination. The neuroanatomical distribution of these reductions also mirrors the recent work from the ENIGMA Bipolar Disorder consortium, which is the largest study of cortical thickness in BD to date. However, whether thinner cortices in BD is the result of abnormal corticogenesis or emerges early in the illness course remains to be clarified. Studies of cortical thickness in non-affected family members of individuals with BD suggest that the illness' genetic risk is associated with subtle thickness abnormalities in frontotemporal cortices. However, a twin study did not find an association between genetic liability for BD and reduced cortical thickness, but rather that unique environmental factors related to BD were associated with cortical thinning in right frontal, limbic, and occipital cortices. Further longitudinal studies are therefore needed to fully clarify the temporal profile of cortical thinning in BD.

We found reduced cortical thickness in BD type II primarily in bilateral dorsolateral, ventromedial, and dorsomedial prefrontal cortices, right perigenual anterior cingulate cortex, and left temporal cortices. Current neural models of mood regulation highlight the importance of neural circuits involving lateral and medial prefrontal cortices, temporal cortices, and subcortical structures. Functional neuroimaging studies suggest that medial prefrontal
cortices are involved in automatic subprocesses of mood regulation, whereas lateral prefrontal cortices are recruited for voluntary regulation.\textsuperscript{7,9} Previous studies found that patients with BD show abnormal activity in medial prefrontal cortices during emotion processing, emotion regulation and reward anticipation and processing.\textsuperscript{46} Furthermore, studies found that modulation of dorsolateral prefrontal cortical activity improved cognitive control and emotion regulation in MDD.\textsuperscript{47} Moreover, activity in the ventral perigenual anterior cingulate and ventral parts of medial prefrontal cortices are linked to reward processing in control subjects, and to anhedonia and amotivation in depressed individuals.\textsuperscript{48} Parts of the medial prefrontal cortex, including the subgenual anterior cingulate cortex, have been linked to experience and regulation of dysphoric emotion in healthy subjects, and show abnormal activity patterns in subjects with depression.\textsuperscript{9,48} Furthermore, activity in this region was found to predict treatment outcome in unipolar depression.\textsuperscript{49} We therefore emphasize the observed effect of recurrent depressive episodes on accelerated thinning in ventromedial prefrontal cortices, including the left subgenual anterior cingulate cortex, in the present study, although this finding did not survive correction for comparisons across the brain. Also, patients with many depressive episodes had significant accelerated thinning in a left temporal region including the superior temporal sulcus and visual areas in the inferior temporal cortex, which provide sensory input to ventromedial and ventrolateral prefrontal networks, forming circuits that support emotional processing.\textsuperscript{48} Anterior and lateral temporal regions are activated during reappraisal,\textsuperscript{50} and lesions of these areas can lead to emotional disturbances.\textsuperscript{51} Altogether, these findings indicate that reduced frontotemporal cortical thickness is one potential neurobiological substrate for mood dysregulation in BD type II.

We found an effect of depressive episodes on cortical thinning in the left temporal difference cluster, which remained significant after adjustment for hypomanic episodes, while an effect of hypomanic episodes was no longer significant after adjustment for depressive
episodes. Despite these findings indicating that only depressive episodes were associated with increased left temporal cortical thinning, we also ran explorative whole-brain analyses for the effects of hypomanic episodes on cortical thickness change. The results indicated that patients with few, but not patients with many hypomanic episodes had increased thinning rate in bilateral temporal cortex clusters and in a right ventromedial prefrontal cortex cluster relative to HCs. This finding was unexpected since previous longitudinal studies found that episodes of mania or hypomania in BD type I were associated with temporal GM loss and frontal cortical thinning. We therefore examined whether patients with few hypomanic episodes had more depressive episodes than patients with many hypomanic episodes, yet found no difference (Supporting Table S3). However, we cannot rule out the possibility that the depressive episodes were more severe in the former patient subgroup and could underlie the increased cortical thinning. The results of these exploratory analyses should therefore be considered cautiously and further longitudinal studies are needed to clarify the effects of hypomanic episodes and episode severity on cortical thinning.

Some limitations of the current study warrant notice. First, we included a modest number of participants, which limits the power to detect subtle structural changes over time. In particular, the association between ventromedial prefrontal thinning and larger number of depressive episodes did not remain significant after corrections for multiple testing and further longitudinal studies are needed to confirm this finding. Second, the attrition rate was ~20% in both groups. We cannot rule out that missing subjects may differ from participants who completed follow-up examinations; however, we replicated group differences in cortical thickness at baseline in the longitudinal subsample, similar to previously published results from the total sample. Third, the number of mood episodes between baseline and follow-up examinations was based on self-report and assessed retrospectively, and was thus prone to recall bias and inaccuracy. We therefore dichotomized this covariate in the main analyses.

In conclusion, the present study provides additional evidence for reduced cortical thickness in regions implicated in mood regulation in BD type II, and the first preliminary longitudinal evidence linking depressive episodes to increased cortical thinning in mood disorders.
However, we also ran analyses for absolute number of mood episodes, but found no significant effects on cortical thickness change. Fourth, normal structural cortical changes may differ between young (where frontal cortex maturation is still taking place) and middle-aged individuals, suggesting that the biological interpretation of the cortical thickness phenotype is not invariant to the age of the sample. However, since the patients and controls in the present study were adults with a similar age distribution, this should not have a major impact on the observed group differences. Fifth, there has been a concern that a cluster-forming threshold of <.05 might be too low. We therefore reran the analyses using a cluster-forming threshold of <.01, and found that main group differences remained significant. Sixth, a large majority of the patients was medicated at both examinations and further longitudinal studies are therefore required to elucidate the effects of psychotropic drugs on cortical thickness. Seventh, we did not include comparison groups of patients with BD type I or MDD, which limits the specificity and generalizability of our findings. Further studies are thus needed to elucidate whether the observed effects on cortical thinning are specific for BD type II or an effect of mood episode-related mechanisms regardless of underlying disorder. Finally, future studies could adopt designs with better control over possible confounding variables, such as duration and severity of mood episodes and medication use, e.g., with cortical thickness examinations during a depressive episode and then after remission. However, based on the findings of the present study, one depressive episode might not cause detectable cortical thickness changes.

In conclusion, the present study provides additional evidence for reduced cortical thickness in regions implicated in mood regulation in BD type II, and the first preliminary longitudinal evidence linking depressive episodes to increased cortical thinning in mood disorders.
ACKNOWLEDGMENTS

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FINANCIAL DISCLOSURES

OAA, UFM, EB, and TE have received speaker's honorarium from Lundbeck. The other authors declare no conflict of interest.
REFERENCES


Zak N. et al.

TABLES

TABLE 1. Demographic and Clinical Characteristics of Individuals With Bipolar Disorder type II and Healthy Controls in the Cross-Sectional Study at Follow-up

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<th>Bipolar Disorder Type II (n=36)</th>
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MADRS; Montgomery-Asberg Depression Rating Scale. YMRS; Young Mania Rating Scale. BD; Bipolar disorder.
Continuous variables are reported as mean ± SD, whereas categorical variables are reported as n (%).

aMissing for one subject with bipolar disorder type II.
bFamily history of bipolar disorder in first-degree relatives. Data was missing for one individual with bipolar disorder type II.
cNo psychotropic medication at least one month prior to examination.
dMood stabilizers at T2 were lamotrigine (n=18), lithium (n=2), carbamazepine (n=1), and topiramate (n=1).
eAntidepressants were citalopram (n=1), escitalopram (n=3), bupropion (n=4), sertraline (n=1), venlafaxine (n=1), mirtazapine (n=2), and mianserin (n=1).
fAntipsychotic agents were quetiapine (n=4) and perphenazine (n=1).
### Table 2. Demographic and Clinical Characteristics of Individuals With Bipolar Disorder Type II and Healthy Controls of the Longitudinal Sample

<table>
<thead>
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<td><strong>Age, years</strong></td>
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<td><strong>MADRS score</strong></td>
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<td><strong>Illness duration, years</strong></td>
<td>17.5 ± 6.9</td>
<td>13.4 ± 6.3</td>
<td></td>
</tr>
<tr>
<td><strong>Social phobia</strong></td>
<td>9 (31.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Panic disorder</strong></td>
<td>13 (44.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>General anxiety disorder</strong></td>
<td>2 (6.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Family history of BD</strong></td>
<td>3 (10.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Years between scans</strong></td>
<td>2.3 ± .47</td>
<td>2.4 ± .13</td>
<td></td>
</tr>
<tr>
<td><strong>Medication</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Unmedicated</strong></td>
<td>9 (31.0)</td>
<td>9 (31.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Mood stabilizers</strong></td>
<td>12 (41.4)</td>
<td>15 (51.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Antidepressants</strong></td>
<td>10 (34.5)</td>
<td>8 (27.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Benzodiazepines</strong></td>
<td>3 (10.3)</td>
<td>3 (10.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Antipsychotics</strong></td>
<td>3 (10.3)</td>
<td>4 (13.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Methylphenidate</strong></td>
<td>0 (0)</td>
<td>1 (3.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Zopiclone</strong></td>
<td>0 (0)</td>
<td>1 (3.4)</td>
<td></td>
</tr>
<tr>
<td><strong>No psychotropic drugs during follow-up</strong></td>
<td>3 (10.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No. of depressive episodes during follow-up</strong></td>
<td>3.0 ± 2.8</td>
<td>3.0 ± 2.8</td>
<td>3.0 ± 2.8</td>
</tr>
<tr>
<td><strong>No. of hypomanic episodes during follow-up</strong></td>
<td>5.0 ± 5.7</td>
<td>5.0 ± 5.7</td>
<td>5.0 ± 5.7</td>
</tr>
<tr>
<td><strong>Psychiatric hospitalization during follow-up</strong></td>
<td>5 (17.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Suicide attempt during follow-up</strong></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>ECT during follow-up</strong></td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Alcohol abuse</strong></td>
<td>0 (0)</td>
<td>2 (6.9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Substance abuse</strong></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Cigarette smoking</strong></td>
<td>6 (20.7)</td>
<td>4 (12.1)</td>
<td></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>25.6 ± 4.4</td>
<td>26.0 ± 4.6</td>
<td>23.5 ± 3.3</td>
</tr>
</tbody>
</table>

MADRS; Montgomery-Asberg Depression Rating Scale. YMRS; Young Mania Rating Scale. BD; Bipolar disorder.

Continuous variables are reported as mean ± SD, whereas categorical variables are reported as n (%).

aMissing for one subject with bipolar disorder type II at follow-up.
bFamily history of bipolar disorder in first-degree relatives.
cNo psychotropic medication at least one month prior to examination.
dMood stabilizers at baseline were lamotrigine (n=11), lithium (n=1), and valproate (n=1), whereas mood stabilizers at follow-up were lamotrigine (n=13), lithium (n=1), carbamazepine (n=1), and topiramate (n=1).
eAntidepressants at baseline were citalopram (n=1), escitalopram (n=5), bupropion (n=1), sertraline (n=1), mirtazapine (n=2), and fluoxetine (n=1), whereas antidepressants at follow-up were citalopram (n=1), escitalopram (n=2), bupropion (n=3), sertraline (n=1), venlafaxine (n=1), mirtazapine (n=2), and mianserine (n=1).
fBenzodiazepines at baseline were oxazepam (n=2) and nitrazepam (n=1), whereas benzodiazepines at follow-up was oxazepam (n=3).
gAntipsychotic agents at baseline were quetiapine (n=2) and flupentixol (n=1), and quetiapine (n=4) at follow-up.
hOne patient could not provide valid information on number of depressive and hypomanic episodes.
Table 2. Demographic and Clinical Characteristics of Individuals With Bipolar Disorder Type II and Healthy Controls of the Longitudinal Sample

<table>
<thead>
<tr>
<th></th>
<th>Bipolar Disorder</th>
<th>Healthy Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>n</em> = 29</td>
<td><em>n</em> = 33</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.3 ± 6.8</td>
<td>35.5 ± 7.0</td>
</tr>
<tr>
<td>Females</td>
<td>20 (69.0%)</td>
<td>18 (54.5%)</td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–10 years</td>
<td>2 (6.9%)</td>
<td>1 (3.4%)</td>
</tr>
<tr>
<td>11–13 years</td>
<td>6 (20.7%)</td>
<td>9 (31.0%)</td>
</tr>
<tr>
<td>14–17 years</td>
<td>12 (41.4%)</td>
<td>9 (31.0%)</td>
</tr>
<tr>
<td>17+ years</td>
<td>9 (31.0%)</td>
<td>10 (34.5%)</td>
</tr>
<tr>
<td>Handedness, right</td>
<td>28 (96.6%)</td>
<td>33 (100%)</td>
</tr>
<tr>
<td><strong>Follow-up</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–10 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11–13 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14–17 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17+ years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Handedness, right</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### MADRS; Montgomery-Asberg Depression Rating Scale. YMRS; Young Mania Rating Scale. BD; Bipolar disorder.

Continuous variables are reported as mean ± SD, whereas categorical variables are reported as *n* (%).

*aMissing for one subject with bipolar disorder type II at follow-up.

*b*Family history of bipolar disorder in first-degree relatives.

*c*No psychotropic medication at least one month prior to examination.

*Mood stabilizers at baseline were lamotrigine (n=11), lithium (n=1), and valproate (n=1), whereas mood stabilizers at follow-up were lamotrigine (n=13), lithium (n=1), carbamazepine (n=1), and topiramate (n=1).

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*e*Benzodiazepines at baseline were oxazepam (n=2) and nitrazepam (n=1), whereas benzodiazepines at follow-up was oxazepam (n=3).

*f*Antipsychotic agents at baseline were quetiapine (n=2) and flupentixol (n=1), and quetiapine (n=4) at follow-up.

*g*One patient could not provide valid information on number of depressive and hypomanic episodes.
Table 3. Clusters Showing Thinner Cortices in Patients with Bipolar Disorder type II Relative to Healthy Controls at Follow-up in the Cross-Sectional Sample

<table>
<thead>
<tr>
<th>Cluster</th>
<th>No. of Vertices in Cluster</th>
<th>Cluster $P$ Value</th>
<th>Regions Within Cluster$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Frontal Cluster</td>
<td>19262</td>
<td>&lt;.0001</td>
<td>Superior frontal gyrus, rostral middle frontal gyrus, pars opercularis of the inferior frontal gyrus, lateral orbitofrontal cortex, frontal pole</td>
</tr>
<tr>
<td>Left Temporal Cluster</td>
<td>9174</td>
<td>&lt;.0001</td>
<td>Superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, temporal pole</td>
</tr>
<tr>
<td>Right Frontal Cluster</td>
<td>16303</td>
<td>&lt;.0001</td>
<td>Superior frontal gyrus, rostral middle frontal gyrus, rostral anterior cingulate cortex</td>
</tr>
</tbody>
</table>

$^a$The cortical regions of the Desikan-Killiany atlas 27 were employed.
Table 3. Clusters Showing Thinner Cortices in Patients with Bipolar Disorder type II Relative to Healthy Controls at Follow-up in the Cross-Sectional Sample

<table>
<thead>
<tr>
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<th>No. of Vertices in Cluster</th>
<th>Cluster $P$ Value</th>
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</tr>
</thead>
<tbody>
<tr>
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<td>19262</td>
<td>$&lt;.0001$</td>
<td>Superior frontal gyrus, rostral middle frontal gyrus, pars opercularis of the inferior frontal gyrus, lateral orbitofrontal cortex, frontal pole</td>
</tr>
<tr>
<td>Left Temporal Cluster</td>
<td>9174</td>
<td>$&lt;.0001$</td>
<td>Superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, fusiform gyrus</td>
</tr>
<tr>
<td>Right Frontal Cluster</td>
<td>16303</td>
<td>$&lt;.0001$</td>
<td>Superior frontal gyrus, rostral middle frontal gyrus, rostral anterior cingulate cortex</td>
</tr>
</tbody>
</table>

$^a$The cortical regions of the Desikan-Killiany atlas were employed.

Table 4. Longitudinal Comparisons of Cortical Thickness Changes

<table>
<thead>
<tr>
<th>Cluster</th>
<th>No. of Vertices in Cluster</th>
<th>Cluster $P$ Value</th>
<th>Regions Within Cluster $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Temporal Cluster</td>
<td>8000</td>
<td>$&lt;.0001$</td>
<td>Superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, banks of the superior temporal sulcus, lateral occipital cortex, fusiform gyrus</td>
</tr>
<tr>
<td>Left Temporal Cluster</td>
<td>5600</td>
<td>$&lt;.0013$</td>
<td>Superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, fusiform gyrus</td>
</tr>
<tr>
<td>Left Parietal Cluster</td>
<td>4626</td>
<td>$.0353$</td>
<td>Postcentral gyrus, supramarginal gyrus</td>
</tr>
</tbody>
</table>

BD; Bipolar disorder. HCs; Healthy controls.

$^a$The cortical regions of the Desikan-Killiany atlas were employed.
FIGURES

Figure 1. *P* value maps for effects surviving cluster-wise correction for multiple comparisons at the .05 level. (A) Regions showing thinner cortices (blue-cyan color) in patients with bipolar disorder (BD) type II compared to healthy controls (HCs) at follow-up. (B) Regions showing significant longitudinal cortical thinning (blue-cyan color) in HCs. (C) Regions showing significant longitudinal cortical thinning (blue-cyan color) in subjects with BD type II. (D) Increased rate of cortical thinning (blue-cyan color) in BD type II compared to HCs from baseline to follow-up. (E) Boxplot showing rate of cortical thickness change (mm/year) within the left temporal difference cluster (indicated by arrow in (D)) for HCs, patients who experienced few depressive episodes between baseline and follow-up (≤2) and patients who had many depressive episodes (≥3). The horizontal lines in the boxes indicate median values. Asterisks indicate significant differences in mean rate of cortical change (*P*<.05; **P**<.001).
FIGURES

Figure 1. P value maps for effects surviving cluster-wise correction for multiple comparisons at the .05 level.

(A) Regions showing thinner cortices (blue-cyan color) in patients with bipolar disorder (BD) type II compared to healthy controls (HCs) at follow-up.

(B) Regions showing significant longitudinal cortical thinning (blue-cyan color) in HCs.

(C) Regions showing significant longitudinal cortical thinning (blue-cyan color) in subjects with BD type II.

(D) Increased rate of cortical thinning (blue-cyan color) in BD type II compared to HCs from baseline to follow-up.

(E) Boxplot showing rate of cortical thickness change (mm/year) within the left temporal difference cluster (indicated by arrow in (D)) for HCs, patients who experienced few depressive episodes between baseline and follow-up (≤ 2) and patients who had many depressive episodes (≥ 3). The horizontal lines in the boxes indicate median values. Asterisks indicate significant differences in mean rate of cortical change (* P < .05; ** P < .001).
**Figure 2.** (A) Corrected $P$ value maps showing regions with increased rate of cortical thinning in mm/year (blue-cyan color) in patients who experienced many depressive episodes between baseline and follow-up compared to patients who experienced few depressive episodes. (B) Corrected and (C) uncorrected $P$ value maps showing regions with increased rate of cortical thinning in mm/year (blue-cyan color) in patients who experienced many depressive episodes between baseline and follow-up compared to healthy controls. Corrected maps show effects surviving cluster-wise correction for multiple comparisons at the .05 level.
Figure 2. (A) Corrected $P$ value maps showing regions with increased rate of cortical thinning in mm/year (blue-cyan color) in patients who experienced many depressive episodes between baseline and follow-up compared to patients who experienced few depressive episodes. (B) Corrected and (C) uncorrected $P$ value maps showing regions with increased rate of cortical thinning in mm/year (blue-cyan color) in patients who experienced many depressive episodes between baseline and follow-up compared to healthy controls. Corrected maps show effects surviving cluster-wise correction for multiple comparisons at the .05 level. (C) Boxplot showing rate of cortical thickness change (mm/year) within a right temporal difference cluster (indicated by arrow in (B)) for HCs, patients who experienced few hypomanic, and patients who had many hypomanic episodes. The horizontal lines in the boxes indicate median values. Asterisks indicate significant ($P<.05$) differences in mean rate of cortical change.

Figure 3. (A) Corrected $P$ value maps showing regions with increased rate of cortical thinning in mm/year (blue-cyan color) in patients who experienced few hypomanic episodes between baseline and follow-up compared to HCs. (B) Corrected $P$ value maps showing regions with increased rate of cortical thinning in mm/year (red-yellow color) in patients who experienced few hypomanic episodes between baseline and follow-up compared to patients who experienced many hypomanic episodes between baseline and follow-up. Corrected maps show effects surviving cluster-wise correction for multiple comparisons at the .05 level. (C) Boxplot showing rate of cortical thickness change (mm/year) within a right temporal difference cluster (indicated by arrow in (B)) for HCs, patients who experienced few hypomanic, and patients who had many hypomanic episodes. The horizontal lines in the boxes indicate median values. Asterisks indicate significant ($P<.05$) differences in mean rate of cortical change.
Mood episodes are associated with increased cortical thinning: a longitudinal study of bipolar disorder type II

SUPPORTING INFORMATION

SUPPORTING TABLES

Table S1. Demographic and Clinical Characteristics at Follow-up of Patient Subgroups Based on Median Number of Mood Episodes Between Baseline and Follow-up

<table>
<thead>
<tr>
<th></th>
<th>Groups based on number of depressive episodes</th>
<th>Groups based on number of hypomanic episodes</th>
<th>$P$ value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Few (0-2), n=15</td>
<td>Many (3-10), n=13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age, years</td>
<td>37.4 ± 7.3</td>
<td>38.0 ± 4.7</td>
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<td></td>
<td>Females</td>
<td>11 (73.3)</td>
<td>8 (61.5)</td>
</tr>
<tr>
<td></td>
<td>Educational level</td>
<td></td>
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<tr>
<td></td>
<td>0–10 years</td>
<td>0 (0)</td>
<td>1 (7.7)</td>
</tr>
<tr>
<td></td>
<td>11–13 years</td>
<td>4 (26.7)</td>
<td>4 (30.8)</td>
</tr>
<tr>
<td></td>
<td>14–17 years</td>
<td>3 (20.0)</td>
<td>6 (46.2)</td>
</tr>
<tr>
<td></td>
<td>17+ years</td>
<td>8 (53.3)</td>
<td>2 (15.4)</td>
</tr>
<tr>
<td></td>
<td>Handedness, right</td>
<td>15 (100)</td>
<td>12 (92.3)</td>
</tr>
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<td></td>
<td>MADRS score</td>
<td>10.1 ± 6.5</td>
<td>9.8 ± 7.9</td>
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<td></td>
<td>YMRS score</td>
<td>2.4 ± 2.4</td>
<td>2.3 ± 1.9</td>
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<td></td>
<td>Illness duration, years</td>
<td>18.1 ± 7.9</td>
<td>17.7 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>Social phobia</td>
<td>5 (33.3)</td>
<td>3 (23.1)</td>
</tr>
<tr>
<td></td>
<td>Panic disorder</td>
<td>6 (40.0)</td>
<td>7 (53.8)</td>
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<tr>
<td></td>
<td>General anxiety disorder</td>
<td>2 (13.3)</td>
<td>0 (0)</td>
</tr>
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<td></td>
<td>Family history of BD</td>
<td>0 (0)</td>
<td>2 (15.4)</td>
</tr>
<tr>
<td></td>
<td>Years between scans</td>
<td>2.4 ± .5</td>
<td>2.3 ± .3</td>
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<tr>
<td></td>
<td>Medication</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unmedicated</td>
<td>5 (33.3)</td>
<td>4 (30.8)</td>
</tr>
<tr>
<td></td>
<td>Mood stabilizers</td>
<td>8 (53.3)</td>
<td>7 (53.8)</td>
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<td></td>
<td>Antidepressants</td>
<td>5 (33.3)</td>
<td>2 (15.4)</td>
</tr>
<tr>
<td></td>
<td>Benodiazepines</td>
<td>1 (6.7)</td>
<td>2 (15.4)</td>
</tr>
<tr>
<td></td>
<td>Antipsychotics</td>
<td>1 (6.7)</td>
<td>3 (23.1)</td>
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<td>Methylphenidate</td>
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<td>1 (7.7)</td>
</tr>
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<td>Zopicclone</td>
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<td>1 (7.7)</td>
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<tr>
<td></td>
<td>No psychotropics</td>
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<td>0 (0)</td>
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<td></td>
<td>No. of depressive episodes</td>
<td>1.0 ± .9</td>
<td>5.2 ± 2.6</td>
</tr>
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<td></td>
<td>No. of hypomanic episodes</td>
<td>4.1 ± 4.3</td>
<td>6.0 ± 7.3</td>
</tr>
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<td></td>
<td>Psychiatric hospitalization</td>
<td>1 (7.1)</td>
<td>4 (30.8)</td>
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<td>Suicide attempt</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<td></td>
<td>ECT</td>
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<tr>
<td></td>
<td>Alcohol abuse</td>
<td>1 (6.7)</td>
<td>1 (7.7)</td>
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<td></td>
<td>Substance abuse</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Cigarette smoking</td>
<td>1 (6.7)</td>
<td>5 (38.5)</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>25.3 ± 4.5</td>
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</tr>
</tbody>
</table>
Mood episodes are associated with increased cortical thinning: a longitudinal study of bipolar disorder type II

Continuous variables are reported as mean ± SD, whereas categorical variables are reported as n (%).
MADRS; Montgomery-Asberg Depression Rating Scale. YMRS; Young Mania Rating Scale. BD; Bipolar disorder.
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*f* Benzodiazepines were oxazepam (n=3).
*g* Antipsychotic agents were quetiapine (n=4).
*h* During follow-up.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>n</th>
<th>%</th>
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<tbody>
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<td>BMI</td>
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<td>Substance abuse</td>
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<td>Suicide attempt</td>
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<td>Hospitalization</td>
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<td>No. of depressive episodes</td>
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<td>Years between scans</td>
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<tr>
<td>BD</td>
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<tr>
<td>Family history of disorder</td>
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<tr>
<td>General anxiety</td>
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<tr>
<td>Panic disorder</td>
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<td>Social phobia</td>
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<td>Handedness</td>
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<tr>
<td>Educational level</td>
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<tr>
<td>Females</td>
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<tr>
<td>Age, years</td>
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<td>Illness duration, YMRS score</td>
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<tr>
<td>Mood episodes</td>
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</table>

Table S1. Demographic and Clinical Characteristics at Follow-up of Patient Subgroups Based on Family History of Disorder

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>n</th>
<th>%</th>
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</thead>
<tbody>
<tr>
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</table>

SUPPORTING TABLES

Medication

<table>
<thead>
<tr>
<th>Medication</th>
<th>Description</th>
<th>n</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>Antipsychotics</td>
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<tr>
<td>Methylphenidate</td>
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<tr>
<td>Mood stabilizers</td>
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<td>Unmedicated</td>
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<tr>
<td>Antidepressants</td>
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</tr>
<tr>
<td>citalopram (n=1)</td>
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</tr>
<tr>
<td>escitalopram (n=2)</td>
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<tr>
<td>bupropion (n=3)</td>
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<tr>
<td>sertraline (n=1)</td>
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<tr>
<td>mirtazapine (n=1)</td>
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<tr>
<td>Almazerine (n=1)</td>
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<tr>
<td>Benzodiazepines</td>
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<tr>
<td>oxazepam (n=3)</td>
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<tr>
<td>Antipsychotic agents</td>
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<tr>
<td>quetiapine (n=4)</td>
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</table>

During follow-up.
Mood episodes are associated with increased cortical thinning: a longitudinal study of bipolar disorder type II

Table S2. Longitudinal Comparisons of Cortical Thickness Changes

<table>
<thead>
<tr>
<th>Cluster</th>
<th>No. of Vertices in Cluster</th>
<th>Cluster P Value</th>
<th>Regions Within Cluster&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients With few Hypomanic Episodes vs. Many Hypomanic Episodes</strong></td>
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<tr>
<td>Left Temporal Cluster</td>
<td>3440</td>
<td>.0307</td>
<td>Superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus</td>
</tr>
<tr>
<td>Right Temporal Cluster</td>
<td>10280</td>
<td>&lt;.0001</td>
<td>Superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, fusiform gyrus, lateral occipital cortex, inferior parietal cortex, banks of the superior temporal sulcus</td>
</tr>
<tr>
<td><strong>Patients With few Hypomanic Episodes vs. HCs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Temporal Cluster</td>
<td>22705</td>
<td>&lt;.0001</td>
<td>Superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, fusiform gyrus, lateral occipital cortex, superior parietal cortex, inferior parietal cortex, banks of the superior temporal sulcus, supramarginal gyrus, postcentral gyrus</td>
</tr>
<tr>
<td>Right Temporal Cluster</td>
<td>10596</td>
<td>&lt;.0001</td>
<td>Superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, inferior parietal cortex, fusiform gyrus</td>
</tr>
<tr>
<td>Right Frontal Cluster</td>
<td>5242</td>
<td>.0023</td>
<td>Lateral orbitofrontal cortex, medial orbitofrontal cortex, frontal pole, superior frontal gyrus, rostral division of middle frontal gyrus, rostral division of anterior cingulate cortex</td>
</tr>
</tbody>
</table>

BD: Bipolar disorder. HCs: Healthy controls.
<sup>a</sup>The cortical regions of the Desikan-Killiany atlas<sup>1</sup> were employed.
Mood episodes are associated with increased cortical thinning: a longitudinal study of bipolar disorder type II

SUPPORTING FIGURES

Figure S1. P value maps showing regions with reduced cortical thickness (blue-cyan color) in patients with bipolar disorder (BD) type II compared to healthy controls (HCs) at baseline in the longitudinal sample (n=62); (A) P value maps surviving cluster-wise correction for multiple comparisons at the .05 level, and (B) uncorrected P value maps. P value maps showing regions with reduced cortical thickness (blue-cyan color) in patients with bipolar disorder (BD) type II compared to healthy controls (HCs) at follow-up (n=71); (C) uncorrected P value maps, and (D) P value maps surviving cluster-wise correction for multiple comparisons at the .01 level.
Mood episodes are associated with increased cortical thinning: a longitudinal study of bipolar disorder type II

**Figure S2.** P value maps showing regions with significantly different rate of cortical change in BD type II compared to HCs; (A) uncorrected P value maps, and (B) P value maps surviving cluster-wise correction for multiple comparisons at the .01 level. Blue-cyan color indicates increased thinning rate, while red-yellow color indicates reduced thinning rate, in patients compared to HCs. (C) Thinning rate in mm/year in HCs and (D) in BD type II. The boxplots show cortical thickness change in mm/year in the left ventromedial prefrontal difference cluster ((E); indicated by red arrow in (A)), the right temporal difference cluster ((F); indicated by black arrow in (A)) and a right anterior cingulate difference cluster ((G); indicated by pink arrow in (A)) for HCs, patients who experienced few depressive episodes between baseline and follow-up (≤2) and patients who had many depressive episodes (≥3). The horizontal lines in the boxes indicate median values. The asterisk indicates significant difference in mean rate of cortical change (*P<.05).
Mood episodes are associated with increased cortical thinning: a longitudinal study of bipolar disorder type II

Figure S2. (A) Uncorrected P-value maps showing regions with significantly different rate of cortical change in BD type II compared to HCs; (B) P-value maps surviving cluster-wise correction for multiple comparisons at the .01 level. Blue-cyan color indicates increased thinning rate, while red-yellow color indicates reduced thinning rate, in patients compared to HCs.

(C) Thinning rate in mm/year in HCs and (D) in BD type II. The boxplots show cortical thickness change in mm/year in the left ventromedial prefrontal difference cluster (E); the right temporal difference cluster (F); and a right anterior cingulate difference cluster (G) for HCs, patients who experienced few depressive episodes between baseline and follow-up (≤2) and patients who had many depressive episodes (≥3).

The horizontal lines in the boxes indicate median values. The asterisk indicates significant difference in mean rate of cortical change (*P < .05).
Mood episodes are associated with increased cortical thinning: a longitudinal study of bipolar disorder type II

**Figure S3.** Uncorrected $P$ value maps showing longitudinal cortical thinning in patients who experienced (A) few depressive episodes and (B) many depressive episodes between baseline and follow-up. Uncorrected $P$ value maps showing regions with increased rate of cortical thinning in mm/year (blue-cyan color) in (C) patients who experienced few depressive episodes between baseline and follow-up compared to HCs and (D) in patients who experienced many depressive episodes between baseline and follow-up compared to patients who experienced few depressive episodes.
Mood episodes are associated with increased cortical thinning: a longitudinal study of bipolar disorder type II

Figure S4. Uncorrected $p$ value maps showing longitudinal cortical thinning in patients who experienced (A) few hypomanic episodes ($\leq 3$) and (B) many hypomanic episodes ($\geq 4$) between baseline and follow-up. (C) Uncorrected $p$ value maps showing regions with reduced rate of cortical thinning in mm/year (blue-cyan color) in patients who experienced few hypomanic episodes between baseline and follow-up compared to HCs. (D) Uncorrected $p$ value maps showing regions with increased rate of cortical thinning in mm/year (red-yellow color) in patients who experienced few hypomanic episodes between baseline and follow-up compared to patients who experienced many hypomanic episodes between baseline and follow-up. (E) Uncorrected $p$ value maps showing regions with increased rate of cortical thinning in mm/year (blue-cyan color) in patients who experienced many hypomanic episodes between baseline and follow-up compared to HCs.
Mood episodes are associated with increased cortical thinning: a longitudinal study of bipolar disorder type II

Figure S5. 
- Figure S5A: Longitudinal thickness changes in patients with few hypomanic episodes between baseline and follow-up, uncorrected.
- Figure S5B: Longitudinal thickness changes in patients with many hypomanic episodes between baseline and follow-up, uncorrected.
- Figure S5C: Longitudinal thickness changes in patients with few hypomanic episodes vs. healthy controls, uncorrected.
- Figure S5D: Longitudinal thickness changes in patients with many vs. patients with few hypomanic episodes, uncorrected.
- Figure S5E: Longitudinal thickness changes in patients with many hypomanic episodes vs. healthy controls, uncorrected.

P value maps showing longitudinal cortical thinning in (A) healthy females, (B) healthy males, (C) females with bipolar disorder (BD) type II and (D) males with BD type II. P value maps showing regions with sex differences in cortical thinning in the total sample. (E) Uncorrected P value maps showing regions with sex differences in cortical thinning in healthy controls. (F) Uncorrected P value maps showing regions with sex differences in cortical thinning in individuals with BD type II. There were no significant sex differences in cortical thickness change after correction for multiple comparisons.
Mood episodes are associated with increased cortical thinning: a longitudinal study of bipolar disorder type II

**Figure S5.** *P* value maps showing longitudinal cortical thinning in (A) healthy females, (B) healthy males, (C) females with bipolar disorder (BD) type II and (D) males with BD type II. *P* value maps showing longitudinal cortical thinning in females vs. males. Blue color indicates increased cortical thinning in females compared to males, while red color denotes more cortical thinning in males compared to females. (E) Uncorrected *P* value maps showing regions with sex differences in cortical thinning in the total sample. (F) Uncorrected *P* value maps showing regions with sex differences in cortical thinning in healthy controls. (G) Uncorrected *P* value maps showing regions with sex differences in cortical thinning in individuals with BD type II. There were no significant sex differences in cortical thickness change after correction for multiple comparisons.
Mood episodes are associated with increased cortical thinning: a longitudinal study of bipolar disorder type II
Mood episodes are associated with increased cortical thinning: a longitudinal study of bipolar disorder type II

**Figure S6.** P value maps showing the significance of the relationships between absolute number of mood episodes and changes in cortical thickness in patients with bipolar disorder (BD) type II. Blue color indicates increased cortical thinning with increasing number of mood episodes, while red color denotes less cortical thinning with greater number of mood episodes. **(A)** Uncorrected P value maps showing regions with significant effect of number of depressive episodes on cortical thickness change. **(B)** Uncorrected P value maps showing regions with significant effect of number of depressive episodes on cortical thickness change, adjusted for absolute number of hypomanic episodes. **(C)** Uncorrected P value maps showing regions with significant effect of number of hypomanic episodes on cortical thickness change. **(D)** Uncorrected P value maps showing regions with significant effect of number of hypomanic episodes on cortical thickness change, adjusted for absolute number of depressive episodes. There were no significant effects after correction for multiple comparisons.
Mood episodes are associated with increased cortical thinning:
a longitudinal study of bipolar disorder type II

SUPPORTING REFERENCES
