Stimulus-specific Synaptic Plasticity in Bipolar II Disorder

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A study using visual evoked potentials (VEPs) to explore synaptic plasticity in bipolar II patients and healthy controls.

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Abstract

Background: Bipolar II disorder is a debilitating psychiatric illness with a higher prevalence in the psychiatric population than previously believed. However, little is known concerning the biological underpinnings of the disorder. In recent years, the glutamatergic hypothesis of depression implicating synaptic plasticity and neuronal circuits in the pathophysiology of affective disorders have gained a lot of attention. The current study is a reexamination, with an aim of replicating the results of an initial study which found increased synaptic plasticity in the visual cortex of healthy controls subjects after exposure to prolonged visual stimulation. If this result is replicated, this would contribute in the validation of VEP-methodology as a means of probing synaptic plasticity in human tissue non-invasively. Further aims for the current study is to find differences in synaptic modulation between the patient and control group, and to explore whether severity of depression as measured by MADRS has an impact on the synaptic plasticity of patients. In addition, neuropsychological tests of verbal and visual memory was administered to map cognitive function in the two groups, and explore whether possible neuropsychological impairment had an impact on the measure of synaptic plasticity.

Methods: VEP-recordings were used to probe synaptic plasticity in a total of 33 BP-II patients and 34 healthy control subjects. The participants were screened for previous head injury, a history of neurological or other severe chronic somatic disorders, and pregnancy.

Results: The results demonstrated an increased modulation of synaptic plasticity in the healthy control group. Further results demonstrated a significant difference in synaptic plasticity between the BP-II and control group, and a significant correlation between severity of depressive symptoms and visual synaptic plasticity.

Conclusion: The results from the current study strengthens the validity of the VEP-methodology as an inexpensive and non-invasive method of recording synaptic plasticity in human subjects.

Preface

The research presented in the current work is part of a larger research project on the biological underpinnings of bipolar II disorder at the department of Neuropsychiatry and Psychosomatic medicine, Rikshospitalet – OUS. The study has employed methods as fMRI, EEG and biological samples in this research. My role in the project has been to accompany a number of the patients and controls during the time of testing. I have also administered the EEG-recordings and the neuropsychological assessment on several of the patients and controls. In addition to this, I've analyzed the EEG-recordings using the MATLAB software.

I would like to sincerely thank my supervisors Stein Andersson, PhD and Kjetil Sundet, PhD for invaluable input and academic advice.

I would also like to thank PhD-candidate Torbjørn Elvsåshagen for including me in his research project on bipolar II disorder, and for thorough training in the administration of EEG-recordings. A thank you also goes out to PhD-candidate Torgeir Moberget for an introduction to the MATLAB analysis of VEP-recordings, and long-distance tech-support in times of need.

Table of Contents

Figures and tables

1.0 Introduction

During the last decade we have witnessed an upsurge in research on bipolar spectrum disorders. It seems that bipolar disorders probably has a higher prevalence in the population than previously believed, and the concept of the bipolar spectrum is gradually expanding to include new forms of the disorder. The consequences for those affected by the disorder are comprehensive, and include interpersonal and occupational difficulties, affective symptoms and cognitive impairment. In addition, the disorder is often associated with comorbid conditions such as anxiety disorders and substance abuse. Bipolar spectrum disorders are increasingly being understood as a systemic condition, associated with somatic states such as cardiovascular disease and obesity (Zarate, Singh and Manji, 2006). There is however sparse research concerning the underlying neurobiology of the disorder – despite a high global prevalence of bipolar disorders.

In the last decade, the prevailing hypothesis concerning the pathophysiology of affective disorders has been the so-called «monoamine hypothesis of depression» – which states that a deficiency of the neurotransmitters serotonin and noradrenaline in crucial areas of the brain contribute to the affective symptoms of depression (Popoli, Gennarelli and Racagni, 2002). The focus has however recently shifted to the possibility of a role for synaptic plasticity and neuronal circuits in affective disorders, a so—called «glutamatergic» or «neuroplastic» hypothesis of depression. Several studies on models of depression in animals has supported this notion, indicating that exposure to stressful stimuli impairs hippocampal and neocortical synaptic plasticity as measured by performance on tasks probing these regions of the brain (Christoffel, Golden and Russo, 2011). Synaptic plasticity entails the mechanism by which information is stored and maintained within the brain's individual synapses. This information contributes in guiding the behavior of a given organism, and allows for the adaptation to constantly changing external and internal enviroments. Animal models of depression have demonstrated that prolonged stress leads to a dysregulation of synaptic plasticity, which again leads to an unbalanced connectivity between different areas of the brain (Christoffel et al., 2011). This imbalance in the brain's internal communication might manifest as the pathological behavior we see in an array of psychiatric illnesses, including depression and the bipolar spectrum disorders. Further evidence supporting the idea of synaptic efficacy being pivotal in affective disorders comes from studies demonstrating that antidepressant and mood stabilizing medication increase synaptic plasticity in the rodent brain. It is however difficult to

translate the results from these animal studies to human subjects. Non-invasive assessment methods of cortical synaptic plasticity in humans are rare, as animal models usually rely on electrodes directly implanted in the brain. However, a few recent studies using repetitive visual stimulation have been able to induce plasticity in both visual and auditory cortices in humans. In these studies, the degree of plasticity is being measured by so-called «visual evoked potentials» (VEP), an averaged stimulus-synchronized electroencephalographic signal that essentially reflects post-synaptic potentials in the occipital cortex (Elvsåshagen, Moberget and Bøen et al., 2012). The plasticity induced by repetitive visual stimulation and measured by visually evoked potentials share several key characteristics with the mechanisms underlying the prevailing hypothesis on the molecular basis for memory in humans, the so-called «long-term potentiation» (LTP). Similar to LTP, plasticity of the VEP is long-lasting, stimulus-specific and dependent on the glutamate NMDA – receptor. It seems that the mechanisms of LTP are both necessary and sufficient to explain the plasticity of the VEP (Elvsåshagen, 2012). The VEP in other words, seem to reflect a form of LTP induced by visual stimulation.

In addition to there being a lack of studies on synaptic plasticity in human subjects, there is also sparse research targeting the relationship between synaptic plasticity and bipolar disorders. Several studies have reported links between impaired synaptic plasticity and stress, and this impairment is often expressed as an impairment in LTP in the hippocampus of animals (Holderbach, Clark, Moreau et al., 2007) or as an impaired modulation effect in evoked potentials (either visual ar auditory) in depressed humans (Normann, Schmitz, Fürmaier et al., 2007). One can assume that similar mechanisms of stress as those explored in depression models in animals are implicated in uni- and bipolar depression in humans, both as precipitating and maintaining factors of the disorders. However, bipolar disorders are more widely regarded as systemic diseases, with psychiatric manifestations being the most pronounced symptoms in the clinical picture. As the research on synaptic plasticity and unipolar depression shows promise, there is therefore a current need to map the role that synaptic plasticity may play in bipolar disorders. We can not exclude the possibility that synaptic plasticity plays an important role in the underlying psychopathology in bipolar disease as well as in unipolar depression. As many bipolar patients receive antidepressant treatment similar to that of patients with unipolar depression and several studies have demonstrated that antidepressant medication increases the degree of synaptic plasticity, synaptic plasticity may be an important target of psychotropic treatment in uni- and bipolar

depressions. For these reasons, research exploring the relationship between synaptic plasticity and bipolar disease might be an important contribution in increasing the knowledge of the underlying pathophysiology of bipolar disorders.

In recent years there has been an increased focus on the subtypes of bipolar disorder, especially bipolar II disorder, which is characterized by episodes of depression and hypomania. It has previously been assumed that this is a "milder" form of bipolar I disorder, which is characterized by interchanging episodes of depression and mania, an assumption that is gradually being challenged as research on the disorder progresses. Current knowledge state that bipolar II disorder follows a more chronic course than bipolar I disorder, and that the depressive episodes affiliated with the condition are just as severe as those found in bipolar I (Berk and Dodd, 2005). However, as the knowledge on prevalence and course of illness increases, basic knowledge on the biological underpinnings of bipolar II disorder are showing less progess. There is still an ongoing discussion conserning the nosological division between the subtypes of the bipolar spectrum, and bipolar II patients are frequently included in larger "bipolar" groups in research studies. With this in mind, there is a need for more basic research focused on the segment of bipolar patients diagnosed as bipolar II. Recent efforts in prevalence-studies have exposed the tendency to misdiagnose bipolar II disorder as unipolar depression, as uncovering episodes of hypomania calls for experienced clinicians or supplementary information from people close to the patient. As neuroplasticity has been implicated in an array of psychiatric illnesses, this study hope to contribute to knowledge on the underlying pathophysiology of bipolar II disorder, by exploring the possible role of synaptic plasticity in the condition. Synaptic plasticity might be a target for future research concerning both the diagnosis and treatment of bipolar disorder, as well as other psychiatric illnesses.

The current study and focus for this account, is a reexamination. The original study (administered in 2010/2011), in line with the aforementioned glutamatergic hypothesis of affective disorders, explored whether the synaptic plasticity in the brains of patients who suffer from bipolar II disease differs significantly from the synaptic plasticity in the brains of normal controls as measured by VEP (Elvsåshagen et al., 2012). The current study replicates the initial examination by utilizing the same methodology, and using a partially overlapping sample of patients and controls. The current examination is therefore conceptualized as a validation-study, where the focus of attention will be whether results similar to those in the

initial study will be obtained. This will contribute to the possible validation of the VEP-methodology as a means of probing synaptic plasticity non-invasively in humans.

This account will give a brief introduction to the concept and history of bipolar disorders. It will further explore the underlying mechanisms of the presumed molecular basis of memory and learning, long-term potentiation (LTP). Here, the evidence concerning impaired LTP in animal models of depression, as well as studies on human subjects will be discussed. Thereafter, the current study and the results of this study- will be described.

1.1 Aims and hypothesis

As mentioned in the introduction, the aim of this study is to validate the VEP-methodology as a reliable and consistent means of measuring synaptic plasticity in human subjects. As most studies on synaptic plasticity so far have been done either in vitro in human brain tissue or invasively in animal subjects, validation of the VEP-methodology would facilitate further research on the subject.

The main hypothesis is two-fold. First and foremost, replicating the results from the initial examination demonstrating that healthy control subjects show synaptic plasticity as a modulation of the VEP, is an important aim in this reexamination. In line with this basic assumption, the second and related hypothesis involves finding a significant difference in the degree of plasticity between the patient and control group. This latter result was also found during the initial examination, and indicates that an impairment in synaptic plasticity might play a role in the pathopshysiology of bipolar II disorder.

In addition to the main hypothesis, several other aims can be formulated. The study will explore whether comorbid psychiatric states will affect the possible relationship between bipolar II disorder and synaptic plasticity. In addition, neuropsychological assessment of visual and verbal memory was carried out in both groups, and possible group differences will be addressed. As the VEP is closely related to the process of long-term potentiation, which is in turn implicated in the process of memory and learning, the relationship between synaptic plasticity and visual/verbal memory will be explored.

2.0 Bipolar disorder II

2.1 Historical perspective

The concept of bipolar disorders as a psychiatric disorder has its diagnostic roots in the theories of Greek physicians during the classical period (Angst and Marneros, 2001). Mania and melancholia are in fact two of the earliest described «abnormal» human conditions. A possible link between the two conditions was also described surprisingly early; by Arateus of Cappadocia in the 1st century BC. He assumed that mania and melancholia shared the same underlying aetiology, and that mania is a worsening of melancholia. This can, presumably, be considered the first conceptualization of bipolar disorder (Angst and Marneros, 2001). The concept of a common aetiology of mania and melancholy was brought up again during the 19th century by the French psychiatrist Jean-Pierre Falret who termed it *«folie circulaire»*, or «circular insanity» in 1851. This signifies the modern re-birth of the concept of bipolar disorder, and also the first time the disorder was described as a separate entity, a cycle between periods of mania and periods of melancholy (Mondimore, 2005). This notion was echoed in Falret's contemporary Baillarger's concept «double insanity», and the concept spread throughout France and the rest of Europe during this time. At the end of the 19th century, the German psychiatrist Kraepelin, by some characterized as the «father of modern psychiatry» due to his theories on bipolar disorder and schizophrenia, introduced the term «manic – depression».

After Kraepelin there was hardly no nosological developments of the concept of bipolar disorders before it was brought back into the scientific spotlight by the Swiss psychiatrist Angst 70 years later, in 1966. The article *«On the Aetiology and Nosology of Endogenous Depressive Psychosis»* (1966) confirmed and developed the earlier hypotheses by Falret and Baillarger, supporting the notion of a shared underlying aetiology of mania and depression. The concept of a bipolar spectrum was first introduced by Kretschmer and Bleuler during the 1920s (Mondimore, 2005). The original idea was conceptualized as a continuum of manic conditions, and has since been expanded to include several forms of bipolarity, including depression interchanging with hypomania. This was first introduced by Dunner et al. in 1976 (Angst and Marneros, 2001), and this variation has later become known as «bipolar II disorder». In general, family members of bipolar probands often display affective and temperamental symptoms that overlap with the diagnosis of bipolar disorder. With this in mind, the existence of an underlying spectrum phenotype has been suggested (Evans, Akiskal,

Keck et al., 2005). A wide array of mood-related or temperamental traits ranging from mild to severe have been observed in family members of bipolar probands, and a complex relationship between geno- and phenotypes in the expression of the disorder are probable (Evans et al., 2005; Hantouche and Akiskal, 2005). One approach to the concept of bipolar disorder therefore argues that it can be conceptualized as a quantitative genetic trait with a continuous distribution – a so-called polygenetic model (Evans et al., 2005). According to Akiskal's theory, the more extreme the temperamental variation, the greater risk of developing BP (Akiskal and Akiskal, 2007). The core feature of the pathophysiology of bipolar disorder according to this approach is therefore a biologically founded dysregulation of mood. Several studies have supported this notion by showing that various measures of temperament (e.g. TEMPS-A and TCI-125) have the ability to predict the risk of developing a disorder in the bipolar spectrum (Evans et al., 2005). In several studies (1977, 1979), Akiskal et al. demonstrated that 35% of subjects with a cyclothymic temperament (as measured by TCI-125) developed either hypomanic, manic or depressive episodes within three years after initial testing (Evans et al., 2005). Linking variations within the bipolar spectrum to temperament suggests a genetic contribution, seeing that temperament is generally believed to be a stable trait throughout an individual's lifespan. This notion was supported in a study by Evans et al. (2005) who managed to distinguish healthy controls and unaffected relatives of bipolar probands by using the TEMPS-A (Temperament Evaluation of Memphis, Pisa, Paris and San Diego). The family members of bipolar probands had higher scores than healthy controls on the subscales «dysthymia», «cyclothymia», «irritability» and «anxiousness». This result was interpreted as a support for the idea that temperament is a quantitative genetic trait related to a susceptibility for bipolar spectrum disorders (Evans et al., 2005).

The disorder once known as «manic-depressive disorder» in Kraepelin’s day, has in other words now expanded into a spectrum, containing the «classical» version of the disorder, shifting between depression and mania, but also other versions such as bipolar II, where depression interchanges with hypomania. Akiskal have also introduced variations within the BP spectrum such as BP III and VI, and even variations labeled BP I ½, II ½ and III ½ (Angst and Marneros, 2001). These subgroups of bipolar disorder will however not be further elaborated in this account.

2.2 Prevalence

Bipolar II, which will be the focus of this account, is much more common in clinical settings than formerly appreciated (Berk and Dodd, 2005). This «discovery» took place already in the late 80s/early 90s when formal studies of bipolar spectrum disorders were first conducted in Memphis, Pisa and Baltimore (e.g. Akiskal and Mallya, 1987; Manning, Haykal, Connor et al., 1997). The systematic national French EPIDEP-study found that using a broader diagnostic framework that encompasses the «hard» BP-I, BP-II and the «soft spectrum» (BP-III and BP-IV) the bipolar spectrum actually accounted for 65% of major depressive disorder (Akiskal, Akiskal, Lancrenon et al., 2006). Furthermore, BP-II was identified as the most common phenotype of the bipolar spectrum disorders when the diagnostic criteria for the duration of hypomania were lowered from four days to two days (Akiskal, Akiskal, Lancrenon et al., 2006). This notion is supported by Perugi (2004) who estimates that 27% - 62% of all major depressions involve hypomania to some degree, and that this is related to already existing premorbid temperamental characteristics.

2.3 Nosological considerations

In DSM-IV and ICD-10, an episode of hypomania is defined as lasting four days or more. Some argue that this cut-off contributes to the frequent misdiagnosis of BP-II as unipolar depression, and that the diagnostic criteria should be reduced to two days, seeing that many hypomanic episodes may not last as long as four days. A bipolar type 2 ½ has been suggested as a subgroup of BP-II where the episode of hypomania lasts less than four days, and is conceptualized as BP-II with cyclothymia, rapidly interchanging periods of hypomania and depression (Akiskal, HS 2003). There are no indications as per now that DSM-V will change the criteria for hypomanic episodes in BP-II (http://www.dsm5.org/ProposedRevisions/Pages/proposedrevision.aspx?rid=426). In addition to the controversial cut-off criteria for hypomania, several other nosological considerations have been outlined in the literature on BP-II. The degree of comorbidity with anxiety disorders, axis II disorders (borderline personality disorder in particular) and substance abuse disorders and the overlapping comorbidity with bipolar I disease, complicates the validity of the diagnosis of BP-II. However-, genetic-, family- and follow-up studies all suggest that the diagnosis is a separate and distinct diagnostic entity (Berk and Dodd, 2005).

2.4 Current conceptualizations

Depression is usually the presenting symptom of BP-II, and for that reason BP-II is often misdiagnosed as a depressive episode/unipolar depression. Hypomanic episodes are often not considered «abnormal» by the patient experiencing such an episode, and comparative records/collateral information or thorough clinical assessment is often necessary to pinpoint the diagnosis of BP-II (Berk and Dodd, 2005). In addition, comorbid states such as panic disorder (Akiskal HS, Akiskal KK, Perugi et al., 2006) and/or substance abuse (Berk and Dodd, 2005) can further complicate the clinical picture. Comorbidity is the rule rather than the exception when it comes to BP-II (Berk and Dodd, 2005). The diagnostic difficulties associated with BP-II have potentially severe consequences, considering the high suicide risk many patients with BP-II often present. Comparing patients with unipolar depression and bipolar disease II indicates that the latter presents with more atypical features, such as hyposomnia, hyperphagia (overeating), increased psychomotor agitation, feelings of guilt, suicidal thoughts and rejection sensibility. (Berk and Dodd, 2005, Akiskal HS, Akiskal KK, Perugi et al., 2006). Some have questioned the discriminatory validity of atypical depression, as this is usually considered a specifier of major depressive disorder. The key symptoms of atypical depression are more closely related to the bipolar spectrum than depressive disorders (Akiskal et al.,2006). Borderline personality disorder is also a misdiagnosis often applied to patients with BP-II, as borderline features are common in patients with BP-II (Berk and Dodd, 2005). Considering the overlap between borderline PD and BP-II, it has been suggested that borderline PD is in fact a part of an underlying bipolar spectrum (Tai, 2007). The atypical symptomatology contributes to the difficulties of correctly identifying and diagnosing BP-II. Mixed states (when hypomania/mania and depression occur simultaneously) are also more common in BP-II, and this might reflect a core feature of the phenotype, or alternatively reflect a consequence of antidepressant treatment. In addition, BP-II might be a more recurring disease than BP-I, as patients with BP-II have more hypomanic and depressive episodes in total compared to their BP-I counterparts (Berk and Dodd, 2005). While earlier thought to be a less severe version of the «original» bipolar disease, bipolar II disease has been shown to follow a more chronic course – and the depressive episodes are thought to be as severe as those seen in BP-I. In general, complex or mixed symptomatology is more common in the bipolar spectrum compared to unipolar patients (Berk and Dodd, 2005).

2.5 Cognitive impairment in bipolar spectrum disorder

It has long been believed that the cognitive impairments associated with bipolar spectrum disorders are limited to illness episodes, and that these symptoms are secondary to the affective manic and depressive symptoms. However, research in recent years indicates that cognitive symptoms are present also in euthymic periods (Bora E, Vahip S and Akdeniz F, 2008). One recent study found that neurocognitive performance in BP-I and II did not correlate with neither illness duration, nor mania ratings as measured by Young's Mania Rating Scale (YMRS) (Simonsen C, Sundet K, Vaskinn A et al., 2008). Several metastudies on neuropsychological functioning in patients with a bipolar spectrum disorder have concluded that cognitive symptoms that persist into euthymic periods include difficulties with verbal memory, sustained attention and certain aspects of executive function (Landrø and Andersson, 2008). According to a recent study, between 3 and 42% of a group of euthymic bipolar patients performed on the level of the 5% poorest performers in a general population on cognitive tasks. This result might indicate that a subgroup of bipolar patients struggle with significant cognitive impairment (Simonsen et al., 2008).

The focus on cognitive impairment in bipolar disorder is often related to the depressive episodes, but some studies indicate that executive dysfunction is greater during manic periods. This finding might be related to the greater frontal dysfunction manic patients display (Bora et al., 2008). In addition, it can be challenging to test cognitive functions in severely manic patients, so the degree of cognitive impairment in manic periods might be higher than what is reflected in the current literature. In general, specific cognitive symptoms related to either periods of depression or mania haven't received a lot of attention, and research on the subject is often ambiguous. According to one review, some studies did not find significant differences between cognitive symptoms in depression and mania, whereas others conclude that cognitive dysfunction is less severe in depression than in mania (Bora et al., 2008). The same review concludes that there is a positive relationship between the number of manic and depressive episodes respectively, and cognitive impairment. This effect is stronger when it comes to the number of manic episodes, indicating that the cognitive dysfunction is indeed more comprehensive during manic episodes (Bora et al., 2008). There is however consistent evidence that verbal memory and difficulties with sustaining attention have been found in bipolar disorder, irrespective of a given affective episode. In addition, impairments in executive function are displayed also in family members of bipolar probands, suggesting that

executive impairment is one of several possible endophenotypes associated with BP (Bora et al., 2008). Considering that some research indicates that bipolar II disorder follows a more chronic course and have more depressive and hypomanic episodes in total compared to their bipolar I counterparts, one might hypothesize that the cognitive impairments in bipolar II are just as, or even more severe than what is seen in bipolar I disorder. One study examining the neurocognitive profiles of patients with bipolar I or II disorder, found a difference between the two groups concerning both magnitude and the pattern of dysfunction (Simonsen et al., 2008). The research group carrying out the study found a significant difference on IQ as measured by WASI between the bipolar I group and the control group. There was no significant difference between the bipolar II group and either the bipolar I or the control group on this measure. Regarding the neuropsychological assessments, the results demonstrated a significant difference between the two bipolar groups on measures of verbal learning and memory, with the bipolar I group performing significantly worse than the bipolar II subjects. On tests of attention and executive function there were no significant differences between the two bipolar groups, with a percentage ranging from 5 - 36% performing on a level characterized as "clinically significant cognitive impairment" (Simonsen et al., 2008). Taken together, these results indicate that bipolar I patients have more widespread cognitive dysfunction than that found in patients with bipolar II disorder. The BP-II group displayed normal cognitive function on measures of verbal memory, and only mild impairments on certain measures of executive function. The cognitive profile of BP-II patients in this study characterized by intact verbal memory and executive dysfunction in short, corresponds more closely to that of depressive patients in three recent studies than with the cognitive profile of bipolar I patients (Simonsen et al., 2008). Thus, accortding to this particular study it seems that patients with BP-II might be more closely related to patients with unipolar depression than patients with BP-I when it comes to cognitive function. Other studies have however demonstrated significant differences in performance on tests of attention and working memory between bipolar II patients and controls, but no significant differences between bipolar I and II patients (Torrent, Mártinez-Arán, Vieta et al., 2006). Significant differences in performance on tests of attention and executive function between bipolar II patients and conrols have been demonstrated in yet other studies (Andersson, Barder, Hellvin et al., 2008). The discrepancy in these results on neurocognitive function in bipolar II disorder can have several causes, it might be attributed to methodological differences, or alternatively, plural depressive episodes may lead to poorer cognitive function.

In the current study, patients with BP-II and controls are tested in both visual and verbal memory tasks, which will be described in detail under the method segment of this article.

2.6 The neuroanatomy of bipolar spectrum disorders

Bipolar spectrum disorders can be conceptualized as a neurobiological disorder, where psychiatric symptoms constitute a prominent part of the clinical picture (Cerullo, Adler, Delbello et al., 2009). Several recent studies have proposed that bipolar disease might in fact be considered a systemic disease, considering that mortality rates due to medical causes are between 1.5 to 3 times higher in people with bipolar disorder compared to the general population (Leboyer, M et al., in press). Structural examinations using MRI have demonstrated that bipolar disorders is associated with a certain degree of frontal cortical atrophy. In general, imaging studies on bipolar subjects show functional (and possibly structural) changes in areas of the brain associated with the cognitive control of affect and emotions, such as the cingulate cortex and the frontal lobe (Bora et al., 2008). A review of neuroimaging studies on bipolar subjects highlight areas implicated in the regulation of emotional, cognitive and social human behaviour. Considering the symptoms associated with bipolar disorders such as affective instability and impulsivity, one can hypothesize that anterior limbic networks are affected in bipolar subjects as these areas control complex socioemotional behaviour (Strakowski, SM; DelBello, MP and Adler, CM, 2005). Several studies have observed changes in prefrontal, subcortical and medial temporal structures, areas associated with anterior limbic networks, in bipolar disorder when compared to control subjects. "The anterior limbic network" specifically includes the amygdala, thalamus, hippocampus, fornix, anterior cingulate cortex, orbitofrontal cortex, ventrolateral prefrontal cortex, dorsolateral prefrontal cortex and striatum (Strakowski et al., 2005). The frontal and striatal areas of the brain have reciprocal connections with limbic regions, and these connections provide feedback essential in emotional regulation and homeostasis (Cerullo et al., 2009). Intuitively, one can hypothesize that this area might display abnormalities in bipolar subjects. The results from neuroimaging studies on bipolar disorder are heterogeneous, but some general trends can still be identified. Specifically, several studies have reported a decrease in grey matter volume in left superior and middle and right prefrontal subregions. The decrease in these areas were positively correlated with more episodes of either depression or mania, and it is also suggested that people with bipolar disorder experience a greater atrophy in grey matter with aging compared to controls

(Strakowski et al., 2005). Other studies have found increases in activation in parts of the PFC such as the dorso- and ventrolateral prefrontal cortex during cognitive tasks (Cerullo et al., 2009). The prefrontal cortex is among other things associated with emotional control and reward processing, and as previously mentioned, this area has several connections with other regions implicated in emotion and affect such as the amygdala and striatum. It is however important to keep in mind that the prefrontal cortex is a large and heterogenous area (Cerullo et al., 2009). Other functional magnetic studies on bipolar disorder have looked at striatal volume, and found an increase in volume in the two regions that comprise the striatum-; caudate nucleus and the putamen. This finding is however not universal, and as illness chronicity is not related to striatal volume it has been hypothesized that striatal enlargement might be a vulnerability factor for developing bipolar disorder rather than a consequence of the illness (Strakowski et al., 2005). Regarding medial temporal areas, several independent studies have reported an increase in amygdala volume in bipolar subjects relative to healthy controls. As the amygdala is a structure related to emotional regulation, this finding has intuitive face validity. There are no reports of decreased hippocampal volume in bipolar disorder, and it has been argued that a normal hippocampal structure and an increased amygdala size might be considered a neurobiological marker of bipolar disorders (Strakowski et al., 2005). A well replicated finding in neuroimaging studies on bipolar disorder is larger ventrical volume, so-called ventriculomegaly, in bipolar subjects compared to healthy controls. More affective episodes are positively correlated with larger ventrical volume (Strakowski et al., 2005). Seen together, evidence from functional imaging studies support a hypothesis implicating the anterior limbic network in affective disorders. An overactivation of these areas is often seen in bipolar patients, and this surplus of activity may lead to an imbalance in emotional stability. This imbalance may again cause a dysregulation of so-called positive and negative feedback loops, which might be implicated in such extreme mood states as depression and mania represent (Cerullo et al., 2009). Regardless of the appeal of this hypothesis, one must interpret functional imaging studies with care. There are also confounding variables such as psychotropic medication and small sample sizes involved in the results mentioned in this segment (Cerullo et al., 2009). Few neuroimaging studies have been done comparing subgroups of bipolar disorder, and as of today there is no conclusive evidence regarding specific neuroanatomical changes in bipolar II disorder.

In general, conclusive knowledge on cognitive impairment and neuroanatomic dysfunction in BP-II disorder is sparse. This may partially be due to the previously mentioned ongoing

discussion concerning diagnostic characteristics of the disorder, and partially due to the fact that the majority of research on bipolar disorder has focused on BP-I. The need for basic research on bipolar disorder is therefore pressing – especially considering the assumed tendency to underdiagnose this particular subtype of bipolar disorder.

3.0 Synaptic plasticity

3.1 Molecular mechanisms

The assumption that information is stored in the brain as changes in the efficacy of synapses was first introduced in the late 19th century by the Spanish pathologist Ramon y Cajal, who theorized that neurons communicate in the junctions known as *synapses (*Bliss and Collinridge, 1993). A synapse is a specialized structure that facilitates the exchange of information between neurons (Christoffel et al., 2011). The axons, who communicate by exchanging information through the synaptic cleft, are either inhibitory or excitatory. The cell body itself (the soma) works as a ”summating device” that adds up the different depolarizing effects coming from various input-signals (Müller et al., 1995). These effects usually last only 5-10 msec, but if a stream of signals occurs simultaneously, the effects of the signals will accumulate. When the total strength of the depolarizing potential in the soma supercedes a certain threshold (around 10 mV), the neuron will fire (Müller et al., 1995).

In 1949 Donald Hebb refined the early ideas of y Cajal, and introduced the concept of ”synaptic plasticity”. This theory is built on the notion that the inherent strength of a synapse is not rigid, and the strength of a synaptic connection can be modified if the level of activity is altered (Müller et al., 1995). This mechanism of synaptic plasticity in the structure of neural connectivity introduced by Hebb, laid the ground for future research on the subject of synaptic transmission and memory and learning (Popoli et al, 2002, Bliss and Collinridge, 1993). The *«Hebbian theory»* describes how there is an increase in synaptic efficacy when the presynaptic cell repeatedly stimulates the postsynaptic cell. The theory can be summarized as «cells that fire together, wire together» (Bliss and Collinridge, 1993). Long-term potentiation (LTP) and it's opposite long-term depression (LTD) represent further developments of the Hebbian theory, and are the most studied physiological models of memory today (Cooke and Bliss, 2006). Long-term potentiation involves the result of a short period of simultaneous pre-

and postsynaptic activity, which in turn leads to the storage of memory traces (Popoli et al. 2002).

The underlying chemical process of LTP was first demonstrated by the Norwegian physician and professor of physiology Lømo and the British physician Bliss (Cooke and Bliss, 2006), in the brain of anaesthetized rabbits (Bliss and Lømo, 1973). More specifically the phenomenon was demonstrated in the granule cells of the hippocampus (Bliss & Lømo, 1973). Since that time LTP has been studied in the brain of rats, mice and monkeys among other species, in areas of the brain ranging from the neocortex to the spinal chord. Considering LTP's strong ties with memory storage, the focus of study is often the hippocampus, as this is a region of the brain strongly implicated in the processes of memory and learning. A structure in the hippocampus, the gyrus dentate, has especially been a focus for study, and this very same structure has also been identified as a site for cell proliferation/neurogenesis, the process where stem cells differentiate to neurons (Sahay & Henn, 2007). The process of neurogenesis has been linked to the pathophysiology of depression, as acute and chronic stress is believed to impair the birth and survival of new neurons (Malberg, 2004). The method of studying human LTP has traditionally been in vitro stimulation of slices taken from the hippocampus. The HC-slides are kept artificially alive in cerebrospinal fluid, and are directly stimulated by electrodes to induce LTP (Bliss & Lømo, 1973). Such HC-slides have allowed for detailed dissection of the molecular mechanisms facilitating long-term potentiation (Ehlers, 2012).

There are several defining characteristics associated with LTP, foreshadowed by Hebb in 1949, that are thoroughly established today. First and foremost, LTP is an *input-specific process*, meaning that a single neuronal pathway can be potentiated without neighbouring pathways being affected by the same stimulation (Cooke and Bliss, 2006). If this form of specificity reflects synaptic specificity, then one can hypothesize that the entity for information storage is in fact one single synapse (Cooke and Bliss, 2006).

A second defining characteristic of LTP is the so-called *«property of associativity»*, which entails that a weak stimulation/tetanus that in itself is not sufficient to initiate LTP, can be potentiated through association with a stronger stimulation/tetanus. This attribute of LTP is relevant to the formation of memory traces, as it represents a cellular analogue of classical conditioning (Bliss & Collinridge, 1993). Third, LTP is characterized by *«cooperativity»*, meaning there is an intensity threshold that needs to be exceeded for LTP to be induced. Weak tetanic stimulation that only activate few afferent fibers, does not lead to the induction

of LTP, and long-term low-tetanic stimulation of afferent fibres can actually lead to a depotentiation, so - called long-term depression (LTD). This mechanism, conceptualized as LTPs opposite, might have a homeostatic function, by controlling that the synapses in the CNS aren't «saturated» by learning. Alternatively, LTD might in itself mediate learning in the form of adaptive forgetting or extinction (Clapp, Hamm, Kirk et al., 2012; Cooke and Bliss, 2006).

The molecular mechanisms of LTP are today quite established and well-known (Cooke and Bliss, 2006). In animal studies, the process of inducing LTP includes electrically stimulating afferents by using high-frequent bursts. Long-term potentiation is observed as a consequence of this stimulation, as an immediate and long-lasting increase in in the post-synaptic response at glutamate synapses in cortical neurons (Clapp et al., 2012). The amino acid glutamate is the major excitatory neurotransmitter in the CNS (Sanacora; Treccani and Popoli, 2012). The electrical stimulation leads to an increased excitability of the dendritic spines in the postsynaptic neuron. This will in turn activate N-methyl-D-aspartate (NMDA) receptors, a form of glutamate receptor that is usually dormant. In addition to activating the NMDA-receptors, the excitability in the dendritic spines of the postsynaptic neuron will also activate voltage-dependent calcium channels that increase the level of calcium ions post-synaptically (Clapp et al., 2012). This transient increase of the concentration of calcium ions induces LTP by activating a cascade of molecular signals that in turn leads to the introduction of another type of glutamate receptors; AMPA – receptors (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid). This subtype is, in contrast to the NMDA subtype, always active. In addition, the ionic conductance of already existing AMPA – receptors will improve. The consequence of this is a larger degree of postsynaptic excitatory responses (Clapp et al., 2011). To sum up; if the intracellular calcium (Ca2+) level reaches a concentration threshold, and this activation lasts sufficiently long, LTP will be triggered (Popoli et al, 2002).

3.2 Long-Term Potentiation and Memory

A large body of evidence suggests that LTP and memory share several similar molecular mechanisms. It is generally assumed that memory functions involve the adaptation of synaptic strength in networks of connected cortical neurons (Clapp et al., 2011). LTP involves a rapid increase of synaptic strength, and as of today represents the best candidate for the neural substrate of memory (Clapp et al, 2011; Bliss and Collinridge, 1993).

Support for LTP being a neural substrate of memory comes mainly from studies on rodents. One study discovered that the blocking of the NMDA receptor impairs learning in rodents exposed to hippocampus-dependent tasks such as the «Morris water maze», a spatial learning task (Cooke and Bliss, 2006). In this particular study, a NMDA-antagonist was infused into the rat's hippocampus. The antagonist did not, however, impair spatial learning once the task was learned by the rats. Similarly, the drug had no effect on the potentiated response once LTP had been induced (Cooke and Bliss, 2006). Other studies on rodents have focused on creating a so-called genetic «knock-out mouse» where the rodents survive well into adulthood, but lacks NMDA receptors located in the CA1 subfield of the hippocampus. These mice faile to exhibit LTP at synapses in the CA1 field of the hippocampus, and in general they displaye specific impairments related to spatial learning and memory, common consequences of hippocampal dysfunction (Cooke and Bliss, 2006). In addition there are some common molecular traits in the intracellular signal mechanisms that mediate LTP and memory; two of the major calcium-responsive pathways (CaMKII and cAMP) play a role in both memory and LTP (Cooke and Bliss, 2006).

It may seem, in other words, that NMDA-dependent LTP plays an important role in hippocampus-dependent learning and memory in rodents. Can these results be translated to human memory function? For obvious reasons, research on human subjects is limited, as few non-invasive in vivo research paradigms exist. Some in vitro studies on the subject have been done by using brain tissue extracted from the hippocampus of patients undergoing surgery for epilepsy. This tissue is kept alive in cerebrospinal fluid, and exposed to electrical stimulation/tetanic bursts. These «in vitro» studies of human tissue have concluded that both NMDA-receptors and cAMP-dependent signaling pathways plays a role in human LTP (Clapp et al., 2012). It has however proven difficult to induce LTP in the brain tissue of epileptic patients with hippocampal epileptic foci. The reason for this might be that the synapses in the epileptic tissue have been potentiated through epileptic activity, and are «saturated» (Cooke and Bliss, 2006). LTP can however be readily induced in tissue from patients with an extrahippocampal epileptic foci, and the LTP displays properties identical to those seen in non – human subjects (Teyler, Hamm, Clapp et al., 2005). Other evidence for human LTP comes from studies that introduce the NMDA-antagonist ketamine in human subjects while learning a verbal memory task (Cooke and Bliss, 2006). The NMDA-receptor blocking Ketamine leads to poor results on the memory task, which suggests that similar to LTP in rodents, the NMDA-receptor also plays an important role in human LTP.

4.0 Synaptic plasticity and stress/depression

For a long time, the predominant hypothesis concerning the pathophysiology of depression has been the so-called «*monoamine hypothesis of depression*» (Musazzi, Racagni and Popoli, 2011). This hypothesis states that depressive symptoms may partially be caused by a deficiency of the neurotransmitters serotonin and noradrenaline in crucial areas of the brain (Popoli, Gennarelli and Racagni, 2002). The monoaminergic systems are distributed throughout the brain in limbic, striatal and prefrontal neuronal circuits, and these circuits are believed to contribute to the behavioral and visceral manifestations of affective disorders (Schloesser, Huang, Klein et al., 2008 and Zarate, Singh and Manji, 2006). The notion of a monoamine hypothesis of depression was brought up by the fact that antidepressant drugs increase extracellular monoamine concentrations by blocking their re – uptake into the cell (Castrén, 2005). In other words, antidepressants, like the SSRIs (selective serotonin re-uptake inhibitors), up-regulate the concentration of serotonin at sites in the brain that play a role in affective regulation. This upregulation of neurotransmitters alleviates the depressive symptoms, and hence might play a role in the underlying pathophysiology of the disorder. For more than half a century this hypothesis has dominated research and guided treatment of affective disorders. While the monoamine hypothesis has been an important contribution to the knowledge on the neurobiological underpinnings of depression, and obviously plays a part in mediating the depressive symptoms of the disorder, the hypothesis falls short of explaining the episodic nature of the mood disturbance associated with bipolar disorders. In addition, even though the initial effects of antidepressant medication are a result of the increase of intrasynaptic levels of serotonin and norepinephrine – the clinical antidepressant effects are observed only after chronic administration (days to weeks). This temporal discrepancy suggests that the monoamine hypothesis has some severe inconsistencies (Sanacora et al., 2012). In addition, research on the effects of antidepressant drug action demonstrate that the efficacy of the medication is less than ideal, with remission rates around 65% and placebo rates reaching 40% in most trials (Henn and Vollmayr, 2006). This raises the question regarding whether the antidepressants available today are hitting the most effective ”target”?

Recent evidence demonstrates that antidepressants and mood stabilizers exert major effects on signaling pathways that regulate cellular plasticity (Zarate et al., 2006). It has therefore been suggested that cascades of downstream events, consecutive series of chemical reactions, might be responsible for the long-term effect of antidepressants (Schloesser et al., 2008).

Hence, in recent years, the focus has shifted from a hypothesis implicating a neurotransmitter deficiency to a hypothesis known as the *neurotrophic*, *glutamatergic* or *neuroplastic* hypothesis of mood disorders, that implies the role of synapses and neural circuits in the pathophysiology of depression (Schloesser et al., 2008). Glutamate is today recognized as the major excitatory neurotransmitter in the human nervous system, and mediate a large part of the excitatory transmissions in the brain. The inhibitory transmissions are largely regulated by the neurotransmitter GABA. If the finely tuned balance in this excitatory/inhibitory this system is disturbed, one can hypothesize that this will lead to subsequent changes in synaptic connections and neuronal circuits. The alterations in synaptic circuits coupled with a genetic predisposition or vulnerability to stress, might play a part in psychiatric illness (Sanacora et al., 2002). The roots of the glutamatergic hypothesis can be traced back to the early 1990s, when it was discovered that NMDA receptor antagonists possess antidepressant-like effects in animals. When rodents were exposed to inescapable stress, this impaired the expression of LTP in the CA1-field of the hippocampus, an area with a high density of NMDA-receptors (Trullas and Skolnick, 1990). This led to the assumption that NMDA-receptors might play a role in the modulation of the behavioral depression-like effects observed after exposure to inescapable stress, and that agents which reduce the transmission in the the NMDA-complex could function as antidepressants (Trullas and Skolnick, 1990). The glutamatergic hypothesis has since grown to include and integrate research from a variety of different fields such as intracellular signaling, neuroplasticity, neurogenesis and synaptic function (Sanacora G et al., 2012). For this reason, a more inclusive and accurate term for the hypothesis is «neuroplastic», and in this account the term «neuroplastic hypothesis of depression» will be used. The hypothesis has developed as several studies have implicated that impaired plasticity and the degeneration of neurons might play a role in the aetiology of mood disorders. The neuroplastic hypothesis of depression argues that stress will lead to neuronal atrophy and decreased neurogenesis (the birth of new neurons) in limbic and cortical areas (the hippocampal formation in particular), and that this effect may partially be counteracted by antidepressant and mood-stabilizing medication (Popoli et al., 2002). The antidepressant and mood-stabilizing medication presumably stimulate intracellular signaling pathways that in turn promote neuronal survival. In other words, both stress and antidepressant treatment have opposite but marked effects on both synaptic strength and on molecular affectors such as the NMDA glutamate receptor, which we know to play a role in the regulation of both long-term potentiation and synaptic plasticity (Popoli et al., 2002). Inherent in this neuroplastic

hypothesis is, in other words, the notion that neuroplasticity is biphasic, it can bee both adaptive (as in the effects of physical exercise and antidepressant treatment), and maladaptive (as in the effects of stress and depression) (Sanacora et al., 2012).

The glutamatergic hypothesis has also been employed in the study of other psychiatric disorders such as schizophrenia. The neuroleptic medication used in the treatment of schizophrenia typically targets the so-called positive symptoms, but have little or no effect on the cognitive symptoms accompanying the disorder (Cooke and Bear, 2011). This observation implies that the cognitive symptoms of schizophrenia do not arise due to the monoaminergic imbalance thought to play a part in regard to the positive symptoms. In addition, phencyclidine (PCP) and ketamine - so-called dissociative or hallucinogenic drugs - produce both positive and cognitive symptoms that mimic schizophrenia in healthy adults (Cooke and Bear, 2011). These substances are both NMDA receptor-antagonists, an observation that has led to the glutamate or neuroplastic hypothesis being implicated in the pathophysiology of schizophrenia as well as the affective disorders.

Among studies that support the notion of a neuroplastic hypothesis of depression, research on rodents have demonstrated the powerful effects stressful events have on both the structure and morphology of limbic and cortical areas in the animals' brain (Musazzi, L et al., 2011). These changes include dendritic simplification, reduction of synaptic spines and global volumetric reduction that mimic those observed in human patients suffering from major depression. Studies on rodents have also demonstrated an increased release and transmission of glutamate in areas implicated in affective disorders, such as the hippocampus, amygdala and prefrontal cortex. This increase in excitatory synaptic transmission has been hypothesized to induce the dendritic changes observed in the rodents exposed to stress models. These maladaptive neuroplastic changes will in turn play a part in the volumetric changes observed in depressed subjects (Musazzi et al., 2011). Research on rodents has also found individual differences in stress-response between the animal subjects. In one study, Henke et al. looked at the synaptic efficacy in the dentate gyrus area of the hippocampus after a group of rats had been exposed to inescapable shocks. The rats who failed to display synaptic efficacy after receiving the shocks, were more likely to subsequently develop a gastric ulcer. The rats that did display increased synaptic efficacy were less likely to develop gastric pathology. The results of the study indicate that increased synaptic efficacy might be associated with a stress-coping mechanism, and failure to display synaptic efficacy might represent a vulnerability factor

(Henke, 1989). This might serve as an analogue to a human stress-diathesis model, stating that certain individuals are more vulnerable to stress than others, and more likely to develop a depressive disorder given a genetic predisposition. Other research supporting a neuroplastic hypothesis of depression has indicated that Litium, which is one of the most commonly used therapeutic medications in bipolar spectrum disorders as it alleviates both symptoms of depression and mania, is neuroprotective and has neurotrophic effects in the human brain. In addition, the medication seems to promote neurogenesis in rodents (Zarate et al., 2006). Litium is generally acknowledged as an effective drug treatment of both bipolar I and bipolar II disorder.

The term «neuroplastic» is in other words a broad term that entails the modification of synaptic number and strength, changes in intracellular signaling, variations in neurotransmitter release, and in some areas of the CNS, the generation of new neurons (Schloesser et al., 2008). It is however important to keep in mind that the monoamine hypothesis and the neuroplastic hypothesis of mood disorders are complementary and not mutually exclusive. Depression is most likely a very heterogenous condition with multiple aetiological factors (Henn et al., 2006), and likewise, bipolar spectrum disorders most likely arise as a result of complex interactions between susceptibility genes and environmental factors

The healthy adult brain has the ability to react adaptively to novel experiences and stressors. Both the structure and the function of the brain undergo plastic modifications (Normann et al., 2007). This capacity for change allows humans to adapt to internal and external changes in environment, a fundamentally important ability in survival and reproduction (Schloesser et al., 2008). Studies on rodents have demonstrated that stress decreases the amount of surviving newborn neurons in the hippocampus, while antidepressant treatment increase this number (Normann et al., 2007). This indicates that the process of neurogenesis is very sensitive to the impact of stress. With that in mind, one can hypothesize that depressive episodes might lead to a decrease in cortical synaptic plasticity in the human brain, as depressive episodes are emotionally stressful events. The idea that stressful events may alter an individuals' response to stimuli in the outside world is not new, but now experimental evidence on rodents have taken steps towards confirming this notion. Several studies have demonstrated how exposure to stressful events in turn will lead to a modification of LTP and synaptic plasticity. Foy et al. (1987) exposed rats to a stress paradigm with or without electric shocks delivered to the tail,

and simultaneously induced LTP in the CA1 area of the hippocampus using high-frequent stimulation. When LTP was recorded 30 minutes after the stimulation, the potentiation was significantly reduced in the animals exposed to stress. There were no differences in the baseline responses in the two conditions (Foy, Stanton, Levine et al.,1987).

4.1 Probing the LTP phenomenon non-invasively in human subjects

In recent years, non-invasive methods for inducing human LTP have been explored. Repetitive transcranial magnetic stimulation (TMS) and electroencephalography (EEG) are examples of research paradigms that allow non-invasive investigations of LTP in healthy, cortical human tissue (Clapp et al, 2011, Cooke and Bliss, 2006). One study employing transcranial magnetic stimulation demonstrated that when area M1 in the motoric cortex was exposed to TMS, this blocked the ability to consolidate the acquisition of motoric skills without actually impairing the motoric performance (Cooke and Bliss, 2006). Teyler et al. (2005) reasoned that in contrast to LTP-expression in deep brain structures such as the hippocampus, LTP of neocortical synapses might be observable as changes in amplitudes of event – related potentials (ERPs). This would enable a non-invasive assessment of human LTP, where the potentiation itself is induced by repetitive visual or auditory stimulation. Zhang et al. (2000) had already demonstrated this potentiation-effect in the visual system of the developing tadpole (Teyler et al., 2005). The results of the study by Teyler et al. on human subjects demonstrated a potentiation of the visual evoked potential (VEP) after exposure to repetitive visual stimulation, and this result is believed to reflect LTP as the underlying mechanism (Teyler et al., 2005). The research paradigm, which initially used healthy control subjects, was explored further by Normann et al. (2007) who hypothesized that if synaptic plasticity is sensitive to stress, depressed subjects should display a lesser degree of potentiation compared to healthy controls. To test this hypothesis, Normann et al., in concordance with Teyler's earlier research, used visual stimulus-specific averaged EEGs (VEPs) as a model to study plasticity in the human visual cortex. The objective of the study was twofold; first, to induce synaptic plasticity in the visual cortex of living humans. Second, to explore whether this form of plasticity is altered in depression. The patient group all suffered from a severe depressive episode following ICD-10 criteria. The results of the study found that continued visual stimulation induced plasticity in healthy human subjects (Normann et al., 2007). The second main finding concerned the alterations in plasticity in depressed subjects. Patients were compared to age-matched controls, and there were

significant differences between the patient and control group in the amplitudes of the C1 and N1 components of the VEP (Normann et al., 2007). In other words, the depressed patients displayed a lesser degree of synaptic modulation in response to visual stimuli compared to healthy controls. In Normann's study, bipolar patients were excluded from the patient group.

The current study elaborated in this account, explores the neuroplastic hypothesis of depression further by employing a VEP-paradigm to measure and compare plasticity in subjects with bipolar II disorder and healthy control subjects.

4.2 Event-related potentials

Current knowledge states that the connection between neurons, so-called synaptic connectivity, is not rigid and unchangeable but flexible and plastic. Impairments in this mechanism have been implicated in the pathophysiology of various psychiatric disorders, such as depression and schizophrenia. Plasticity in the synapse occurs when the potentiation of the post-synaptic neuron is prolonged. A means to record and measure this prolonged potentiation, is to study brain wave activity. A normal electroencephalogram-recording (EEG) reflects thousands of simultaneous processes in the brain. A more ”specialized” ERP however, finds the average of all the EEG activity that occurs as a response to a given stimulus (Luck, Woodman and Vogel, 2000). When post-synaptic neurons fire, the electrical potentials ”travel” passively through the brain and to the scalp where the signals contribute to the more comprehensive EEG. The EEG is recorded by several electrodes placed on the scalp, and a temporally defined average of these signals are used to extract the smaller ERPs from the larger EEG-recording (Luck, 2005). Any brain activity that’s not relevant to the stimuli at hand, will over a large number of trials average to zero. All brain activity that is within the defined time-frame connected to the stimuli, will lie within the averaged acitivity that comprise the ERP. The ERP-waveform consists of several positive and negative peaks, and these peaks or components are often marked with a ”P” or an ”N” to indicate positivity or negativity (Luck, 2005). The succession of the components that follow a stimuli reflect a fixed sequence of neural processing triggered by a visual stimuli; early sensory processes followed by more complex decision- and response-related processes (Luck et al., 2000). The amplitude and frequencies of the various peaks can be used to study the temporal aspects of cognitive processes. The distribution of voltage over the base of the skull can be used to study the neuroanatomical loci of these processes (Luck et al., 2000). The former is the focus of the current study.

Visual evoked potentials are a form of ERP specific to the visual system. The brain processes visual stimuli in a certain manner, which is reflected in three ERP - components: C1, P1 and N1. C1 is a component that will be induced anytime a visual stimuli is presented. Any paradigm that presents visual stimuli can be used to study the C1-component which usually peaks around 70-100 msec. C1's distribution across the scalp is quite broad, with the greatest polarity at occito-parietal sites contralateral to where the stimuli is presented (Luck et al., 2000). The P1 component which usually peaks around 80 – 130 msec, displays its maximum amplitude over the lateral parietal part of the skull, specifically around the ventrolateral prestriate cortex. The N1 peaks around the time when selective attention comes into play (130 – 200 msec). It has been suggested that the N1-component reflects the allocation of attention resources. It has also been suggested that the N1 might be the manifestation of a sensory "gating-mechanism" for attention (Luck et al., 2000). The VEP is comprised of several components identified as positive or negative 'peaks' in the ERP-waveform, and the three mentioned above are the focus of the present study. The early component (C1) is independent of attention and not related to perception. The subsequent components (P1 and N1) are slightly modulated by attention. These three early components of the VEP are less likely to be influenced by cognitive processes, and more likely to reflect actual synaptic plasticity. As more time passes from the exposure of the stimuli, more advanced cognitive processes come into play. Visually evoked potentials seem to show a great deal of consistency over time (Normann et al., 2007).

In addition to using a photic stimuli, stimulus-related evoked potentials can also be induced by auditory stimuli, usually in the form of tone pips (Cooke and Bear, 2011). Studies using auditory stimuli demonstrate that sensory LTP is not limited to the visual system, auditory evoked potentials (AEPs) recorded in the auditory cortex can be induced by tetanic auditory stimuli (Clapp et al., 2011).

5.0 Initial study

The intention of the current reexamination is first and foremost to replicate the results from the initial study by Elvsåshagen et al. (2012), and thus validating the VEP-methodology as a paradigm for assessing synaptic plasticity in vivo in humans.

The results from the initial VEP – study, which was largely modeled after Normann's study (2007), demonstrated a significant increase in the P1 and N1 amplitudes in healthy control subjects. The most robust effect was however the peak-to-peak measure between the P1 and N1 components. The P1 to N1 peak-to-peak was therefore conceptualized as a possible superior measure of plasticity (Elvsåshagen et al., 2012). The initial study found that the bipolar II group displayed no significant modulation effects on the C1, P1 or N1 component, nor the P1 to N1 peak-to-peak measure. The control group displayed significant modulation effects on the P1, N1 and P1-N1 peak-to-peak measures. There was however no significant modulation of the C1 component. This result overall supports the notion that people with bipolar II disorder displays impairments in synaptic plasticity, in line with the initial hypothesis.

There were however no significant differences in plasticity between healthy controls and medicated patients (antidepressant and mood-stabilizing medication). This result echoes research on rodents where antidepressant and mood-stabilizing medication has been found to mediate synaptic plasticity (e.g. Holderbach et al., 2006). The study found no significant effects between depressive symptoms (as measured with MADRS) and synaptic plasticity. This lack of significant effects between depressive symptoms and synaptic plasticity is however attributed to two outliers, who may have skewed the data material. By removing these outliers from the data material, a significant correlation between severity of depressive symptoms and synaptic plasticity was attained.

6.0 Methods and Materials

6.1 Participants

33 patients with BP-II (mean [SD] age, 35,5 [8,75] years; 19 women) were recruited from psychiatric outpatient clinics in the Oslo area. The sample is partially overlapping with the sample used in the initial examination in 2010. Specifically, 15 patients participated in both the initial examination and the current re-examination. Clinical examinations were carried out by a senior psychiatrist at the department of neuropsychiatry and psychosomatic medicine at Rikshospitalet - OUS, a university department specializing in, among other things, the evaluation and treatment of affective disorders. Axis 1 diagnosis and comorbid states were assessed using the Mini-International Neuropsychiatric Interview (MINI) DSM-IV criteria

version 5.0. (Sheehan et al., 1998). Mood state at the time of VEP-recording was determined using the Montgomery-Asberg Depression Rating Scale (MADRS) (Montgomery SA and Asberg, M, 1979) and the Young Mania Rating Scale (YMRS) (Young, RC et al., 1978).

32 healthy control subjects (mean [SD] age, 35,19 [9,57]; 17 women) matched with the patient group for gender and age were recruited through local advertising. 30 healthy controls participated in both the initial and current examination. The control subjects underwent a full examination similar to the patient group. The exclusion criteria for both groups were practically the same as those applied in the initial examination in 2010: age below 18 or over 55 (in the initial examination the maximum age was 50), previous head injury with loss of consciousness for more than one minute, a history of neurological or other severe chronic somatic disorders, and pregnancy. All subjects had normal or corrected to normal visual acuity. The Regional Ethic Committee of South-Eastern Norway approved the study, and all subjects provided informed consent to participate.

6.3 Data reduction

Six subjects were removed from the material during the process of the EEG-analysis because they consistently displayed divergent VEP – potentials. Four of those cases were patients, and two cases were control subjects. One of these control subjects was removed because of the lack of an identifiable C1-component. Likewise, the C1 components in all phases of the VEP (baseline 1 and 2, poststimulation 1 – 6, see fig.1 for illustration of the paradigm) - was removed from the data material in two subjects, both patients, because there were no identifiable C1-peaks. One control subject was removed from the neuropsychological data material, as the scores on both the visual and verbal task were divergent.

6.2 Experimental paradigm

Visual evoked potentials were evoked by using checkerboard reversals in two baseline blocks: one plasticity-inducing modulation block, and six postmodulating blocks (as shown in fig.1). In the baseline and postmodulation blocks, 40 checkerboard reversals were displayed within a time interval of 20 seconds. In the modulation block, VEPs were evoked by checkerboard reversals for 10 minutes. The baseline blocks were displayed two and eight minutes after the experiment started, and the modulation block was initiated 2 minutes after the last baseline block ended. The postmodulation blocks were initiated 2, 8, 12, 18, 22 and 28 minutes after the modulation block ended. In the intervals between the checkerboard stimulation, a grey

screen was shown. The participants were instructed to focus on a small red circle (.1°) placed in the center of the screen during the entire experiment. The participants were allowed to listen to music. All patients were monitored to ensure that they were focusing on the screen throughout the experiment. Altogether, the experiment lasted 47 minutes. The visual stimuli were presented with E-Prime 1.1 (Psychology Software Tools, Sharpsburg, Pennsylvania) on a Samsung Syncmaster 2493 HM LCD screen (Samsung Electronics Nordic AB, Oslo, Norway).

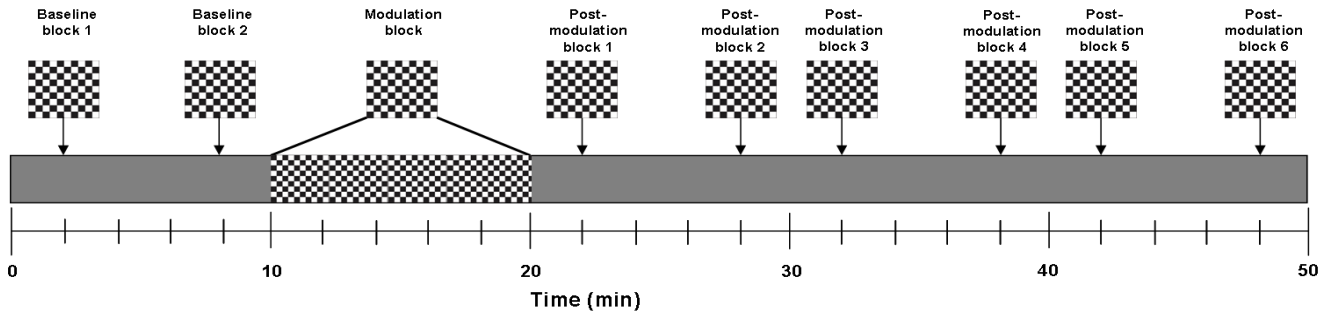

Figure 1: illustration of the VEP-paradigm.

6.4 Recording and Analysis of the VEP

Electroencephalographic signals were recorded from the occipital lobe using three monopolar silver/silver chloride electrodes at O1, O2 and Oz, according to the international 10-20 system. The impedances were kept below 5 kΩ. Ground and reference electrodes were attached to the forehead. Eye movements were recorded using bipolar electrodes placed at the sub – and supraorbital regions at the lateral canthi of each eye. The EEG activity was sampled at 250 Hz with an amplifier band-pass of .05-100 Hz. Offline EEG analysis was conducted with EEGlab, run in MatLab 7.6.0. (MathWorks, Natick, Massachusetts). The EEG-data was first high-pass filtered at 1 Hz, subjected to independent component analysis and segmented into epochs starting 100 msec before and continuing 300 msec after the onset of each cherckerboard stimuli. Blinks were discarded using independent component analysis (values) and thereafter manually revised. The epoched EEG was low-pass filtered at 30 Hz and averaged into two baseline and six post-modulation specific VEPs. The C1 was defined as the most negative peak within 70 – 110 msec after visual stimulation, the P1 as the most positive peak within 90 - 140 msec after stimulation and the N1 as the most negative peak within 130 – 190 msec after stimulation. Amplitudes and latencies for the C1, P1 and N1 component were obtained from the Oz – electrode only. All amplitudes were measured relative to the 100-msec baseline.

6.5 Neuropsychological assessment

The participants in the study were tested on modified versions of the Brief Visuospatial Memory Test – Revised (BVMT – R) (Benedict RHB, 1997) a visual memory task, and the Rey Auditory Verbal Learning Test (RAVLT), a test of verbal memory (Schmidt M, 1996). The tests were expanded to include 12 figures and five trials (BVMT) and 20 nonsemantically or phonemically related words and five trials (RAVLT) respectively. The original tests consist of six figures and three trials (BVMT) and 15 words and five trials (RAVLT). The two neuropsychological tests were modified with the intention of increasing the variability in memory performance among healthy youg adults, the verbal learning test was modified in line with Pereira et al. (2006). During the initial examination no significant differences between patients and controls were observed, as the patient group is a high-functioning group quite similar to the control group regarding their level of education. It was also observed that many subjects, both patients and controls, reached a ceiling effect during the recall-phase of the assessment. The patients and controls were initially tested prior to the VEP – recording, and tested on delayed recall and recognition briefly after the VEP-recording. The time interval between initial testing and the recognition task did not exceed two hours.

6.6 Statistical Analyses

All statistical analyses were conducted with SPSS, version 18.0 for Windows (SPSS, Chicago, Illinois). A two-tailed *p* value of <.05 was considered significant. The categorical data, namely gender and level of education, were correlation-tested using chi-squares. There were no significant differences in the VEP amplitudes between the two baseline recordings, and for this reason these two recordings were combined in the analyses as one baseline/prestimulation variable. Likewise, the VEP amplitudes from the six poststimulation recordings were averaged into one poststimulation variable. A paired-samples t-test was used to explore the effect of the stimulation phase on the poststimulation amplitudes of C1, P1, N1 and P1-N1 peak-to-peak respectively. To obtain scores that reflected the "plasticity-effect" of the stimulation phase on both C1, P1, N1 and the P1-N1 peak-to-peak, baseline VEP amplitudes were subtracted from their corresponding poststimulation amplitudes. These "plasticity"- or "modulation-scores" were subjected to oneway analyses of variance to determine the effects of group (bipolar II versus controls). The impact of severity of depression as measured by MADRS on synaptic plasticity was explored using bivariate correlations. As no patients obtained a clinically significant score on YMRS, this measure

was excluded from the statistical analyses. The correlations between the amplitudes of the VEP on intial and reexamination was explored using bivariate correlation analysis.

The results from the verbal and visual memory tests were compared between groups using independent sample t-tests. The variables explored in the analysis were total learning (trial 1-5), delayed recall, delayed recognition (both number of hits and false positives), and a "learning score" constructed by subtracting the result on trial five from the result on trial one. The relationship between neuropsychological performance and synaptic plasticity was explored using bivariate correlations.

7.0 Results

7.1 Demographic and clinical variables

Table 1 reports demographics and clinical data for patients and healthy control subjects. No significant difference was found between the groups for age [$t(59) = .147$, $p < .844$], gender [$\chi^2(4, N = 61) = 8.13$, $p = .087$], or level of education [$\chi^2(3, N = 61) = 5.88$, $p = .118$] Twenty-one patients were euthymic (defined as MADRS score <11 and YMRS score <8), five patients were mildly depressed (defined as MADRS score (11 - 20), and three patients were moderately depressed (defined as MADRS score 22 - 31). No patients were severely depressed, defined as MADRS score >31. As mentioned, no patients were hypomanic at the time of testing (YMRS score <8). Concerning the use of psychotropic medication, nine patients used only antidepressants, 10 patients used only mood-stabilizing medication, and eight patients used both antidepressants and mood-stabilizing medication. Two patients were unmedicated. None met the criteria for current alcohol or drug abuse, as measured by MINI. Panic disorder without agoraphobia as assessed by MINI was a frequent comorbid psychiatric state (16 patients and one control subject). Other comorbid states were panic disorder with agoraphobia (four patients), social phobia (two patients) and generalized anxiety disorder (one patient).

Table 1: Demographic and clinical variables for patients and controls.

	Bipolar II group (N=29)	Control group (N=32)	χ^2	*p*
Mean age in yrs (SD)	35.52 ± 7.8	34.85±9.5		.883
Females/males	19/10	17/15	1.24	.265
Education level			8.14	.118
0-10 yrs	2 (6.9 %)	-		
11-13 yrs	7 (24.1 %)	3 (9.1 %)		
14-17 yrs	7 (24.1 %)	10 (30.3 %)		
17+ yrs	11 (37.9 %)	20 (60.6 %)		
Missing	2 (6.9 %)	-		
MADRS mean (SD)	8.79 ± 6.8	1.18 ± 1.5		<.001
YMRS mean (SD)	2.41 ± 2.5	.62 ± .1		<.001
Medication				
Unmedicated	2 (6.9 %)			
Mood stabilizers (a)	20 (69.0 %)			
Antidepressants (b)	14 (48.3 %)			
Comorbid states				
Panic disorder without agoraphobia	16 (55.2 %)	1 (3.0 %)		
Panic disorder with agoraphobia	4 (13.8 %)			
Social phobia	2 (6,9 %)			
General anxiety disorder	1 (3.4 %)			

(a) Mood stabilizers were: lamotrigin
(b) Antidepressants were: escilatopram, buproprion, venlafaxin, mirtazapin and sertraline.

7.2. Baseline VEP

The stimulation consisting of checkerboard reversals produced the hypothesized reaction in the form of a VEP-curve in both healthy control subjects and patients, with an average negative baseline amplitude (C1) at 87.69 msec, a positive amplitude (P1) at 114.45 msec and a second negative amplitude (N1) at 150.03 msec after the reversal. There were no difference between healthy control subjects and patients in the baseline C1 (t (57)=-.167, p = .868) and P1 (t (59)= 1.66, p = .102) components, but there was however a significant difference between the groups in the N1 component (t (45)=-2.10, p = .041) and the P1-N1 peak-to-peak component (t (50)=3.44, p = .001) .

7.3. VEP plasticity in healthy control subjects

There were no significant effects of the modulation block on the C1 component *(t = -1.86; p = .071)*. There were however, significant effects in the P1 *(t = -2.665; p = 0.012)*, N1 *(t = -2.753; p =.010)* and P1 – N1 peak-to-peak *(t = -6.957; p = <.001)* amplitudes.

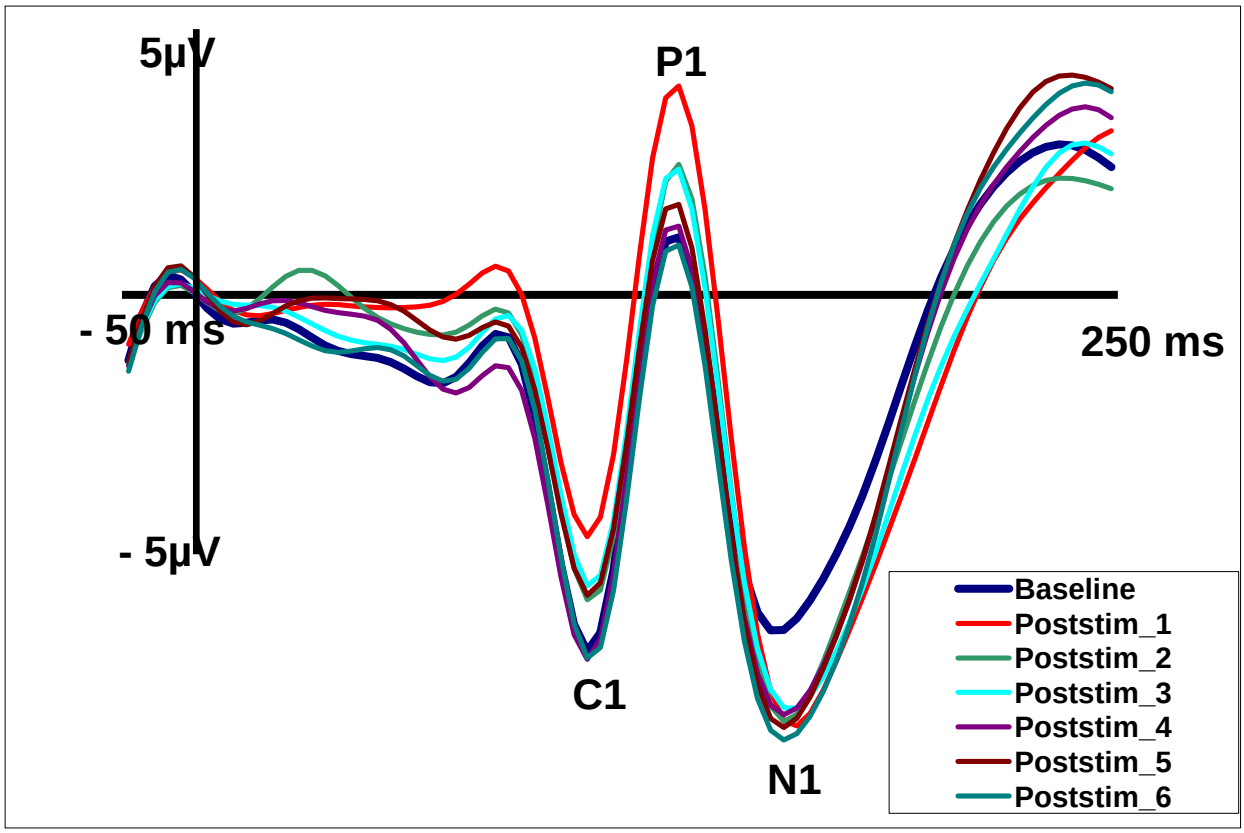

Fig 2: graphic depiction of the averaged VEP-components in baseline block and poststimulation blocks 1-6

7.4. VEP plasticity in patients with BP-II

In the patient group there were opposite effects to those found in the control group. Hence, there was a significant effect of the modulation block on the C1 amplitude *(t = -3.425; p = .002)*, but no significant effects on the P1 *(t = -.993; p = .329)*, N1 *(t = .035; p = .290)* or P1-N1 peak-to-peak *(t = -1.07; p = .290)* amplitudes.

7.5 VEP plasticity in controls versus patients with BP-II

The effects of the modulation block on the VEP amplitudes in healthy control subjects versus the bipolar II-subjects were explored. There was a significant difference in plasticity of the P1-N1 peak-to-peak amplitude [F(1,59) = 8.83; *p* = .004], and a trend towards a significant difference of the N1 amplitude [F(1,59) = 3.78; *p* = .057]. No significant differences were

found on the C1 [F(1,57) = 2.25; *p* = .139] or the P1 [F(1,59) = .58; *p* = .462] amplitudes between the two groups.

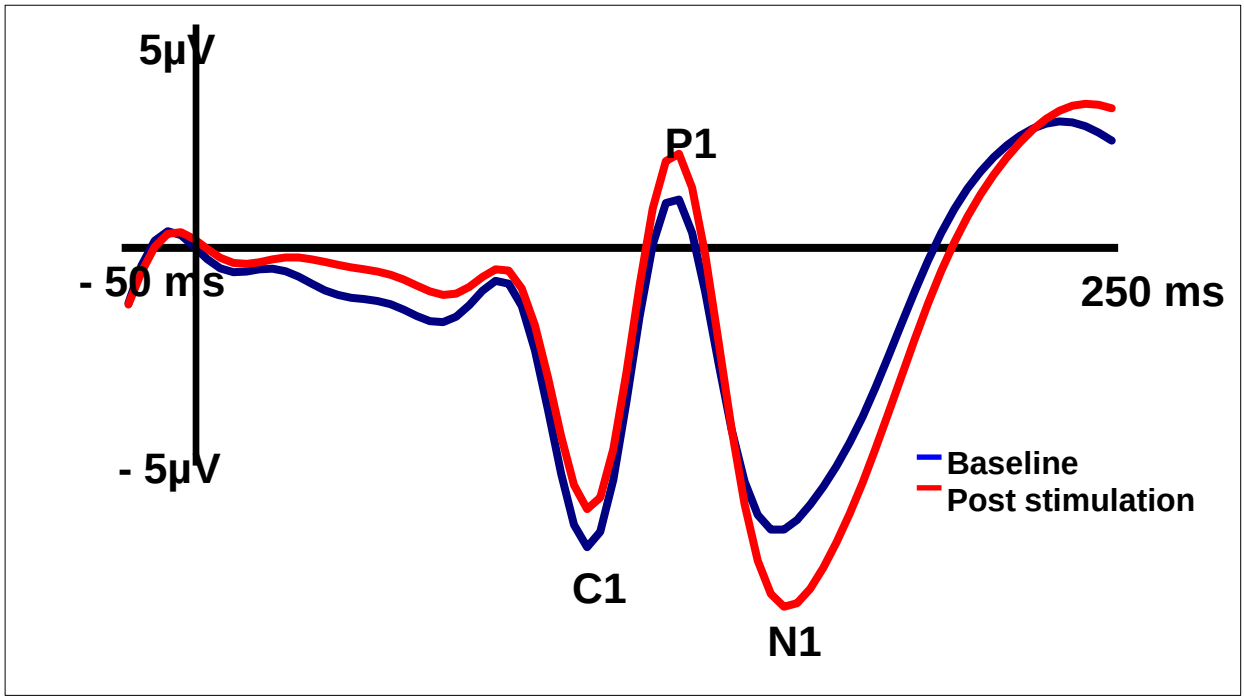

Fig 3: Graphic depiction of the averaged pre- and poststimulation VEP components in healthy control subjects.

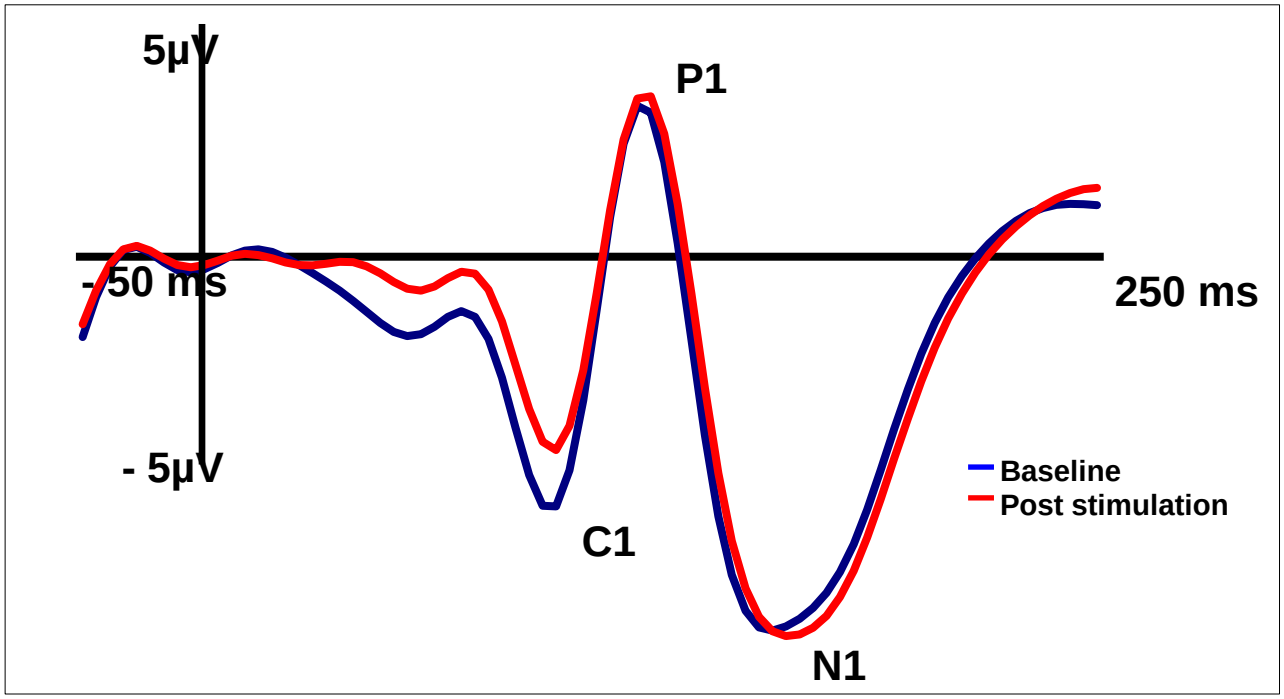

Fig 4: Graphic depiction of the averaged pre- and poststimulation VEP components in BP-II patiens.

7.6 Validity of the VEP-paradigm; comparing initial and current examination

30 healthy controls and 15 patients participated both in the initial examination and the current reexamination. The relationship between the previously mentioned "plasticity scores" on the initial (T1) and current (T2) examination was explored using Pearson product-moment correlation coefficient on C1, P1, N1 and P1-N1 peak-to-peak respectively. The analysis was only conducted on the control subjects, as the main objective was to test whether the VEP-paradigm is a valid measure of synaptic plasticity. The patient group has undergone several changes concerning both medication (more patients were medicated during T1 than T2) and depression (fewer patients were clinically depressed as measured by MINI on T1 compared to T2) from the initial testing to the current one. For these reasons, the control group will be the only focus of the analysis.

There was a strong, positive correlation between the P1 [r = .51, n = 29, p = .004] and the N1 [r = .57, n = 29, p = .001] plasticity components on T1 and T2. There was a trending positive correlation on the C1 plasticity component [r = .33, n = 28, p = .080], but no significant correlation of the P1-N1 peak to peak [r = .067, n = 29, p = .729] plasticity component in T1 and T2. See figure 4 for a scatterplot depicting the amplitudes of patients and controls on C1, P1, N1 and P1-N1 peak-to-peak in T1 and T2.

7.8 VEP plasticity, mood state and psychiatric comorbidity

A bivariate correlation was conducted to explore the impact of severity of depression as measured by MADRS on all modulation scores, C1, P1, N1 and P1-N1 peak-to-peak. There were significant correlations in the relationship between MADRS-score and P1 modulation score (r = -.389, p = .037) and the P1-N1 peak-to-peak modulation score (r = -.404, p = .030). There were no significant correlations between severity of MADRS-score and the C1 (r = .320, p = .104) and N1 modulation score (r = -.033, p = .863). The strenght of the relationships between the significant correlations are small to moderate. Se fig. 5 for a scatterplot presentation of the relationships between severity of depression and plasticity.

Only six patients had an ongoing depressive episode at the time of examination (as assessed by MINI). These six patients overlapped with the patients assessed as mildly or moderately depressed using MADRS. An independent samples t-test was conducted to compare the patients with an ongoing depressive episodes with patients with no ongoing depressive episode on a measure of plasticity. The patients with an ongoing depressive episode (M=-

1.483, SD=3.2) were significantly different from the patients with no depressive episode (M=1.081, SD=2.4, t= (27) -2.161, p=.040). This means that the patients with an ongoing depressive episode displayed a lesser degree of plasticity compared to the patients with no ongoing depressive episode.

Concerning psychiatric comorbidity neither panic disorder with or without agoraphobia nor social phobia had any effect on synaptic plasticity.

7.9 Verbal and visual memory assessment

An independent samples t-test was conducted to compare the performances on a test of visual memory (BVMT) and verbal memory (RAVLT) respectively, for patients and controls. The analysis found a significant difference in the scores between the two groups on the visual memory task regarding both learning (patients; M = 55.6, SD = 23.5, controls; M = 66.7, SD = 17.5, t = (51.6) -2.04, p = .046), delayed recall (patients; M = 15.5, SD = 6.2, controls; M = 18.6, SD = 4.9, t = (53.1) -2.14, s = .036), and one aspect of recognition, 'false positives'; (patients; M = .97, SD = 1.21, controls; M = .29, SD = .783, t = (47.4) 2.58, s = .014). There were no significant differences between the two groups regarding the other aspect of recognition, 'number of hits'. There was a significant difference between the two groups on the so-called "learning score"; (patients; M=11.62, SD=4.41, controls; M=14.34, SD=3.15, t = (50.2) -2.747, p=.008). All of the significant group differences obtained an effect size between .50 - .70 using Cohen's D (Cohen J, 1992). This is considered a medium to large effect size. There were however, no statistically significant differences between patients and controls on any measure of verbal memory, specifically total learning, delayed recall, recognition (hits and false positives) and the aggregated learning score. See table 2 and table 3 for results of the correlation between patients and controls on BVMT and RAVLT respectively.

7.10 Neuropsychological assessment and plasticity

The results on the neuropsychological tasks were correlated with the P1-N1 peak-to-peak plasticity score to explore whether performance on visual and verbal memory tasks were related to synaptic plasticity. There were a few significant correlations, specifically between learning of visual stimuli and P1-N1 peak-to-peak (p = .367, s =. 050) and delayed recall of visual stimuli and the N1 plasticity component (p = .379, s = .043) in the patient group, and between the number of hits in the delayed recognition task and the P1 plasticity component in the control group (p = .362, s = .046).

Table 2: Overview of correlations of BVMT-scores, bipolar II and controls.

BVMT Variable	Bipolar II patients (N=29)		Healthy controls (N=31)		F (df)	Effect size (Cohen's D)	*p*
	M	SD	M	SD			
Total Learning	55.69	23.5	66.71	17.5	7.05 (58)	-0.53	*.046*
Delayed recall	15.52	6.2	18.65	4.9	6.02 (58)	-0.55	*.036*
Delayed recognition/hits	11.38	.82	11.68	.65	3.39 (58)		.123
Delayed recognition/false positives	.97	1.2	.29	.78	10.35 (58)	0.66	*.014*
Learning (T5 ÷T1)	11.62	4.4	14.41	3.1	4.10 (58)	-0.70	*.007*

Table 3: Overview of correlations of RAVLT-scores, bipolar II and controls.

RAVLT Variable	Bipolar II patients (N=29)		Healthy controls (N=31)		F (df)	*p*
	M	SD	M	SD		
Total Learning	65.69	13.11	71.19	12.96	.030 (58)	.101
Delayed recall	13.79	3.83	15.32	3.39	1.04 (58)	.107
Delayed recognition/hits	19.00	1.46	19.29	.97	1.94 (58)	.366
Delayed recognition/false positives	1.41	2.32	1.10	1.68	.381 (58)	.545
Learning (T5 ÷T1)	8.20	2.70	8.35	2.82	.079 (58)	.837

8.0 Discussion

The results of this study produced three main effects. First of all, a significant plasticity effect of the P1, N1 and P1-N1 peak-to-peak in healthy control subjects was observed. Secondly, there was no similar modulation of these components in the bipolar II patients. This echoes the results from both the initial examination, and other previous reports of VEP plasticity in healthy control subjects after exposure to visual stimuli. The C1 component was however not significantly potentiated in the control subjects, but did display a significant plasticity effect in the patients. This finding is similar to that of Normann et al. (2007), who found a significant increase in the P1 and N1 but not the C1 amplitudes in healthy controls using a similar research paradigm (Normann et al., 2007). This early C1 component most likely reflects early sensory processes, which is induced in the human visual cortex anytime a visual stimuli is presente. Another observation echoed in the work of Normann et al. (2007), is that the component displaying the strongest modulation effect in the control subjects, is the N1-component. The reason for this discrepancy in degree of potentiation among the VEP-components is currently unknown. In addition, the patients concistently displayed higher amplitudes on all components of the VEP. This phenomenon was present also in Normann (2007) and Elvsåshagen's (2012) studies. The consistently higher amplitudes in depressed and bipolar II patients might reflect a medication-effect, or other unknown disease-related factors. This was not controlled for in the current study, but might be explored in future research using similar methodology.

The third main effect found was a significant difference between patients and controls on the P1-N1 peak-to-peak modulation, and a trending significance in the N1-component. This finding is consistent with the results from the initial examination, where significant differences in the P1-N1 peak-to-peak was also demonstrated, This result can in other words be regarded as a contribution towards validating the VEP-methodology as a consistent measure of synaptic plasticity in the human visual cortex. The difference in potentiation between controls and patients persisted despite the fact that most of the patients were taking either antipdepressants, mood-stabilizing medication or both, during this re-examination. Only 6.9% of the patient group were unmedicated, and none of the patients displayed severe depressive symptoms. Eight patients were characterized as mildly or moderately depressed using MADRS, while six patients were characterized as having an ongoing depressive episode using MINI. The discrepancy in the number of patients being characterized as

depressive in MADRS and MINI is due to MINI being a diagnostic tool, while MADRS is more of a screening tool for assessing depressive symptoms. The criteria for a depressive episode in MINI is therefore more stringent than those outlined in MADRS. The results demonstrated a significant positive correlation between MADRS score and the P1 and P1-N1 modulation scores. This result indicate that severity of depressive symptoms is related to a greater impairment in synaptic plasticity.

During the initial examination, 38.5% of the patients were unmedicated, and the mean MADRS score was 14.5±9.1, compared to a mean of 8.8±6.8 in the current study. One can therefore hypothesize that the imparment in synaptic plasticity in patients with BP-II is related to the underlying pathophysiology of the disorder rather than being connected to specific affective episodes, as the difference between bipolar patients and controls persist also when a large percentage of the patient group are in euthymic or symptom-free periods. The impairment in synaptic plasticity seems, in other words, to be related to *trait* rather than *state*. The result obtained in the current study can for these reasons be considered more robust than the results from the initial examination, as the two groups were more "similar" during testing in the reexamination, in the sense that fewer patients were depressed and more patients were medicated.

Keeping in mind that the impaired plasticity in BP-II patients seem to be independent of mood state, even though the impairment correlates positively with more severe depressive symptoms, reduced synaptic plasticity might represent a biological marker for bipolar spectrum disorder. Previous electrophysiological studies on bipolar disorders have found significant differences in bipolar patients and controls on the so-called mismatch negativity (MMN) component of ERP. This component occurs when a deviant stimulus (usually differing in frequence or duration) is presented in a line of predictable auditive stimuli (Andersson et al., 2008). The component is thought to represent an automatic response that is preattentive, reflecting change-detection processes in the auditory cortices (Näätänen R, Kujala T, Essera C et al., (2012). The MMN is composed of contribution from both auditory and frontal areas, and seem to be implicated in a number of psychiatric and neurological disorders (Näätänen et al., 2012). Few studies have been done in regard to the MMN-component in bipolar disorders, but there seems to be a significant increase in the latency of MMN in bipolar patients compared to healthy controls. This result, coupled with a reduced frontal MMN amplitude, has also been found in bipolar II patients (Andersson et al., 2008).

These results indicate a possible dysfunction in the fronto-temporal circuitry that's implicated in MMN. The MMN-pattern found in bipolar patients might be a possible electrophysiological marker for bipolar disorder. Further studies are needed to establish a possible electrophysiological "profile" for bipolar patients, where both the MMN and synaptic plasticity might be implicated.

8.1 The impact of antidepressant and mood-stabilizing medication on synaptic plasticity

One would expect that antidepressant and mood-stabilizing medication would have an impact on the degree of plasticity, considering that results from the initial study indicated that the medicated patients displayed a significantly larger degree of plasticity compared to their unmedicated counterparts. This finding has been demonstrated in previous accounts as well, both on humans and rodents (Normann et al, 2007 and Holderbach R, Clark K, Moreau JL, Bischofberger J and Normann C, 2007). Normann et al. (2007) gave the antidepressant sertraline to the healthy control subjects, and observed an increase in plasticity in the P1 and N1 components of the VEP complex. Holderbach et al. (2007) exposed rodents to a three week period of mild stress, and observed that antidepressant medication counteracted the impairment in LTP that the stress paradigm produced (Holderbach et al., 2007). As the neurotransmitters serotonin and noradrenalin is implicated in the modulation of synaptic plasticity, antidepressants might protect neural networks that are vulnerable to stress by increasing the levels of these signal mechanisms (Normann et al., 2007). In the current re-examination, only two of the patients were unmedicated, which makes the background for drawing any conclusions concerning synaptic plasticity and the use of antidepressant or mood-stabilizing medication in this context rather sparse. There were no significant effects of antidepressant or mood-stabilizing treatment on synaptic modulation, but there was however a trend towards a greater degree of plasticity in patients using both antidepressant and mood-stabilizing medication compared to patients only using one of the two groups of medication. This trend was quite modest, but never the less indicates that the combination of several types of medication used in affective disorders might lead to a greater degree of synaptic plasticity than monotherapy.

8.2 Memory assessment and synaptic plasticity

The statistical analysis revealed a significant difference between the patients and controls on almost all aspects of a visual memory test (BVMT). One exception was the number of false

positives generated in the recognition task. There were however no significant differences between the two groups on a test of verbal memory, which is somewhat surprising considering that impaired verbal memory is one of the most consistent findings in research on cognitive dysfunction in bipolar disorder. However it might be that this only applies to patients with bipolar I disease, as a recent study found significant differences in performance on a verbal learning task between bipolar I patients and healthy controls, but no significant differences between the performance of the bipolar II group compared with either of the two other groups (Simonsen et al., 2008). A possible focus for future research is exploring whether impairments in verbal memory is a variable that could contribute to the nosological distinction between bipolar I and bipolar II disorder. The lack of significant differences between the groups on the verbal memory task might also be contributed to the mood state of the patient group, as a majority of patients were euthymic on the time of testing. A study by Martínez-Arán et al. demonstrated that euthymic bipolar patients with more subjective complaints performed poorer on an array of neuropsychological tests compared to euthymic bipolar patients with fewer subjective complaints (Martínez-Arán A, Vieta E, Colom F et al., 2005). This might be interpreted as subjective dissatisfaction influencing objective cognitive impairment, and this is a possible variable not controlled for in this study.

When the neuropsychological test performance was correlated with a measure of synaptic plasticity, only a few and seemingly arbitrary significant correlations were found. The lack of significant correlations is perhaps not surprising, considering that the tasks of verbal and visual memory taps areas of the brain associated with memory and learning, such as the hippocampus and the medial temporal lobe, while the VEP-paradigm primarily taps the visual cortex. One might however presume that the synaptic plasticity observed in the visual cortex, or the lack thereof in the case of the bipolar II patients, can be generalized to other areas of the brain as well. If synaptic plasticity is implicated in the pathophysiology of affective disorders, then an impairment in synaptic efficacy might be found in areas of the brain implicated in mood regulation. Rodent models of depression have found impaired hippocampal synaptic plasticity in animals displaying depressive symptoms (e.g. Holderbach et al., 2007). Brain imaging studies of human subjects have demonstrated that areas of the brain implicated in emotional regulation shows alterations in bipolar disorder. The glutamatergic hypothesis of affective disorder state that impaired synaptic plasticity in these very same areas of the brain might contribute to the clinical symptoms seen in affective disorder. The research on synaptic plasticity in relation to human psychiatric illness is

however still in its preliminary stages, and future research is needed to elaborate on the role that plastic mechanisms might play in affective disorders.

8.3 Test-retest correlations; validity of the VEP-paradigm

The correlations between the modulation scores on the initial examination and current re-examination demonstrated significant correlations on the P1 and N1 modulation scores. This result indicates that the plasticity of the VEP after long-term visual stimulation is a robust phenomenon that may be utilized as a non-invasive paradigm in research on synaptic plasticity in human subjects. There are several advantages associated with using ERP-methods in research as it is an inexpensive, non-invasive and sensitive way of accessing preattentive cognitive functions not readily accessible using neuropsychological testing only (Andersson et al., 2008). The C1 component however did not show a significant correlation from initial to current testing. Considering that this component consistently displays the lowest degree of plasticity in both the current and previous accounts, this result is not that surprising. More puzzling on the other hand, is the lack of significant correlation on the P1-N1 peak-to-peak modulation. This amplitude was found to be the most robust indicator of plasticity both in the initial study, and was suggested as a possible overall measure of VEP plasticity (Elvsåshagen et al., 2012). The current test-retest results weakens this hypothesis, but further studies are needed to explore what variables show the greatest modulation effect. Interestingly, there was a strong correlation between the plasticity of the modulation block in the initial and current examination. In this block VEP plasticity was induced by a 10-minute display of cherckerboard reversals. In addition to displaying a significant effect in the control group on the two examination points, there was also a significant difference between the patient and the control group on this measure. It's worth noting that the plasticity in the modulation phase might be a stronger overall measure of synaptic plasticity than the previously presumed P1-N1 peak-to-peak. If this turns out to be a valid and robust measure of plasticity, it opens the possibility of reducing the overall time used administrating the VEP-paradigm, which in this particular examination lasted a total of 47 minutes.

It is important to keep in mind that an in vivo demonstration of synaptic plasticity in humans using prolonged visual stimulation represents a correlation, and not a causal effect. The current examination can however be considered a contribution towards validating the VEP-paradigm as a non-invasive window into human synaptic plasticity in the visual cortex. In recent years, synaptic plasticity has increasingly been implicated in theories of

pathophysiology in an array of psychiatric illnesses, making stimulus-specific synaptic plasticity an area of research holding a lot of promise. The results from this study also correspond well with the prevailing hypothesis concerning the underlying mechanisms of human depressive illness, the “glutamatergic” or “neuroplastic” hypothesis. This hypothesis implies the role of synapses and neural circuits in affective disorders. Previous accounts, including the initial study preceding this reexamination, have also concluded that an impairment in synaptic plasticity might be related to the clinical picture we see in depressive episodes in bipolar II disorder. Regarding the hypomanic phases of bipolar II disorder, the current study did not allow for exploration of the link between hypomania and synaptic plasticity. The reason for this was that no patients displayed clinically significant hypomanic symptoms. This will however be an interesting approach for further study.

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10 Figures and tables

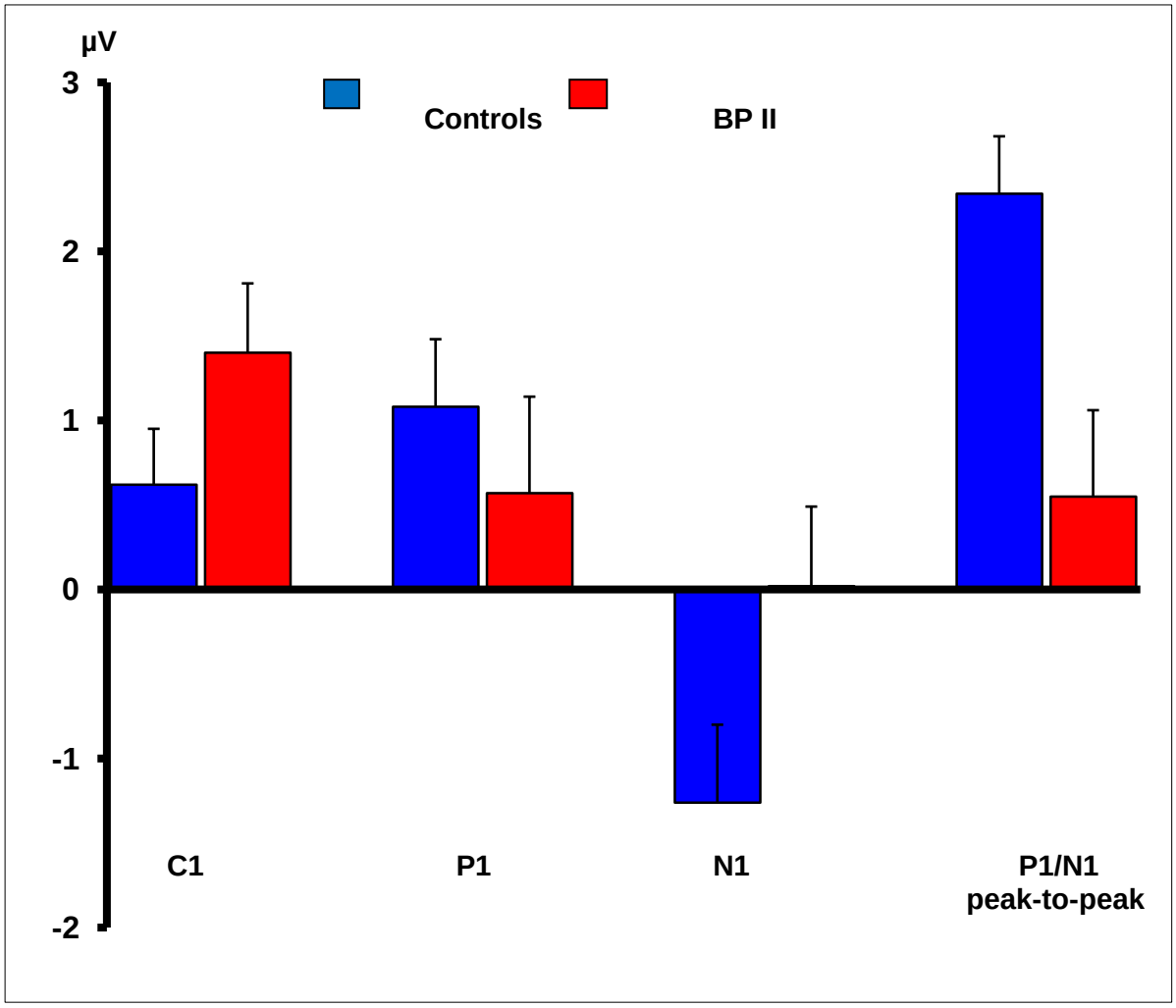

Figure 5: Overview of synaptic modulation on C1, P1, N1 and P1-N1 peak-to-peak in BP-II patients and controls.

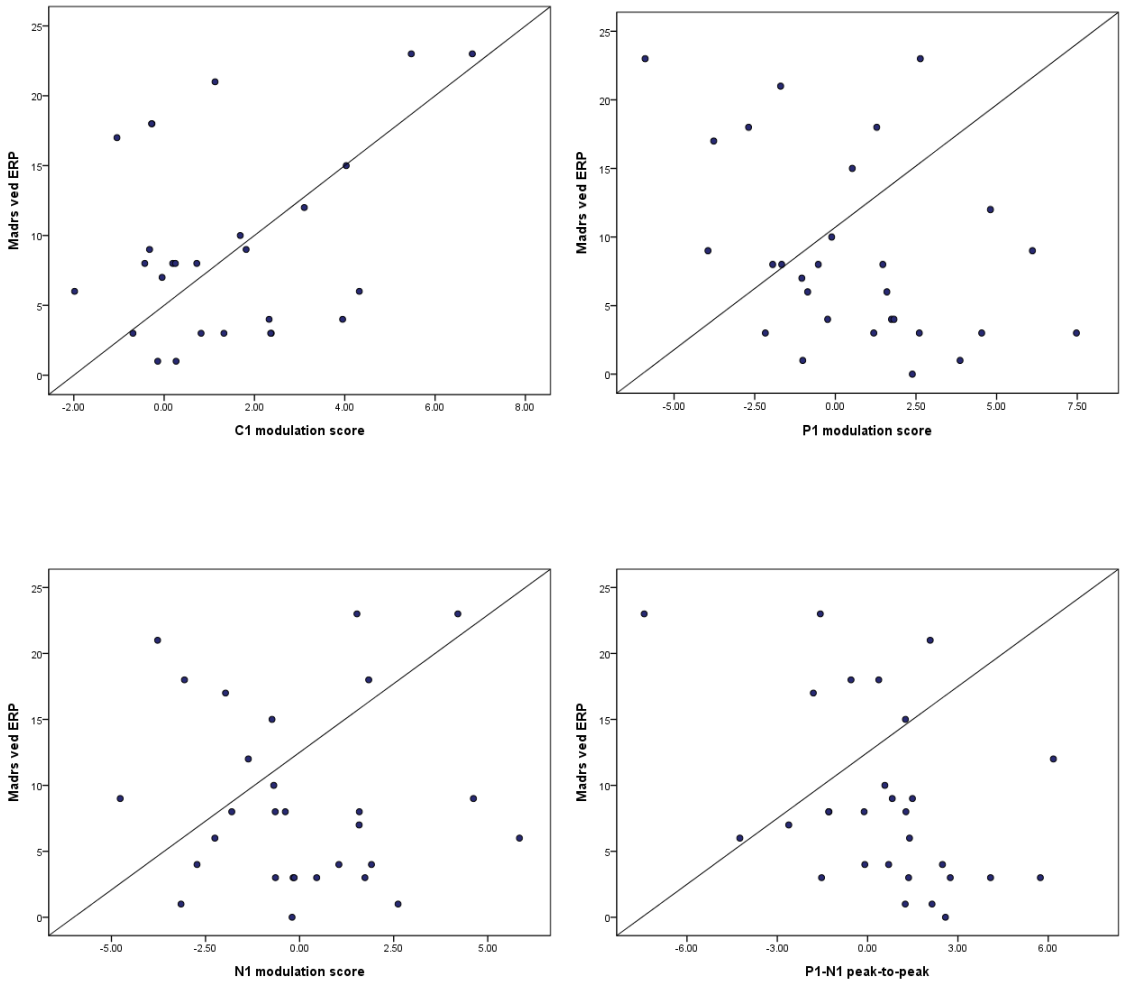

Figure 6: Scatterplot of the correlation between severity of depression as measured by MADRS and synaptic plasticity

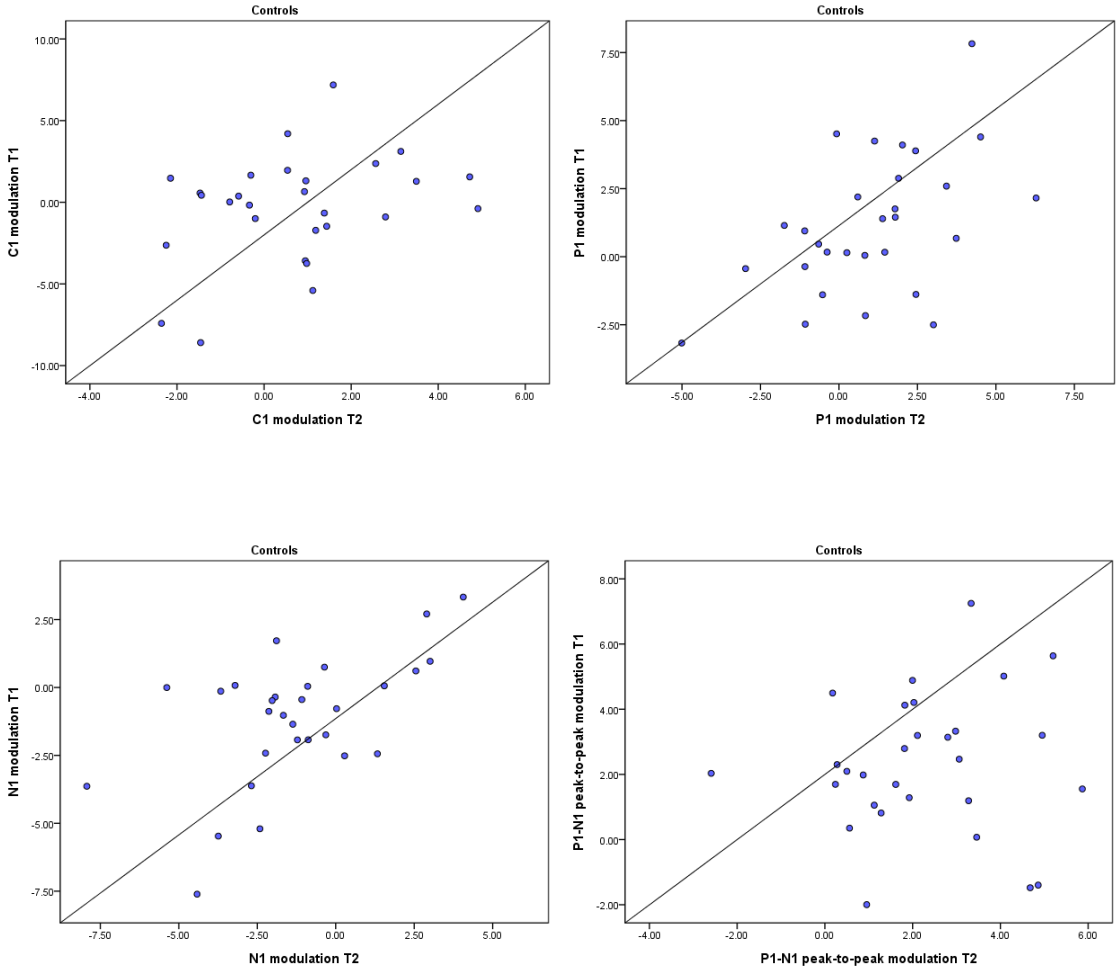

Figure 7: Scatterplot of test-retest correlations of modulation effect on C1, P1, N1 and P1-N1 peak-to-peak in healthy control subjects.