The role of BDNF in depression : will the neurothrophin hypothesis sparkle on, long after the glitter of the firework is gone?

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The Role of BDNF in Depression

Will the neurotrophin hypothesis sparkle on,

long after the glitter of the firework is gone?

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Cover-illustration: *Vuurwerk Scheveningen strand* by Anouk Mentink (2013)

Will the neurotrophin hypothesis sparkle on, long after the glitter of the firework is gone?

Leaving aside the details of the discovery of BDNF by Yves-Alain Barde and his colleagues (1982) (some call it *heroic experimentation* [Reichardt 2006] others *a fortunate accident* [Y-A Barde, personal communication, 2012]), it does have a great legacy. Among this legacy is the *neurotrophin hypothesis*, which states that pathological conditions such as depression (partly) are secondary to an altered expression of BDNF. The theoretical and clinical possibilities of this hypothesis loom(ed) large, yet numerous hurdles are on the path towards definite inferences from it. We are still learning and answers may only come with time, new data, and alternative interpretations of the data that already are out there. This is what I tried to do over the course of the past few years and here I present the results of this exercise. And, do the results favor a sparkling future for the neurotrophin hypothesis? Well, they don't as the findings of this thesis (solid work over novelty) detail inconsistencies on many fronts and make me contemplate that: '*all that glitters is not gold* (William Shakespeare, 1596-1598) – it can be fireworks as well'.

January 2014, The Hague

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CHAPTER 1

GENERAL INTRODUCTION

Neurotrophins

A classic example of the notion that *new cultures often start with great discoveries* is the discovery of Nerve Growth Factor (NGF), in the 1950s by Rita Levi-Montalcini, Stanley Cohen and Viktor Hamburger. Follow-up experiments on NGF, mostly performed by two of its discoverers; Levi-Montalcini and Cohen, convincingly showed that the signaling of this hormone serves at least two important functions: (**I**) the specific survival of neurons from a larger set of neurons (pruning, selective apoptosis) and (**II**) the maintenance of neuronal connections (Cohen *et al.,* 1954; Levi-Montalcini, 1966; Levi-Montalcini, 1987). With these discoveries Levi-Montalcini and Stanley Cohen paved their way to a Nobel Prize (Physiology or Medicine, 1986) and, importantly, to *the understanding of many disease states such as developmental malformations, dementia and their treatment* (the Nobel Committee, 1986).

Soon after its discovery it became apparent that NGF is not unique in its crucial functions for neuronal survival and maintenance but rather that it is a member of a family of related molecules. Subsequently were discovered, in order of appearance, Brain-Derived Neurotrophic Factor (BDNF; Barde *et al*., 1982), Neurotrophin-3 (NT3; Maisonpiere *et al*., 1990) and Neurotrophin 4/5 (NT4/5; Berkenmeier *et al*., 1991), all molecules with similar functions, yet different types of target receptors. Although NGF to date remains the most studied neurotrophin (11,884 published papers of which 4,412 in the past ten years [PUBMED, August 2013]), BDNF (11,751 published papers [only 133 less than on NGF] of which 8,478 in the past ten years [4,066 more than on NGF]) has become a strong competitor in terms of allocation of research efforts devoted to it. The two Benjamin's of the family, NT3 and NT4/5, clearly lag behind, with to date a summed up total of 2,751 published papers (of which 1,178 in the past ten years). So, BDNF related research has been on the rise. This rise may possibly be due to several features that are unique for BDNF, for instance its activity dependent secretion and function as a key regulator of neuronal function.

Brain-Derived Neurotrophic Factor

BDNF is a small dimeric hormone that consists of 247 amino acids with a total molecular weight of 27.8 kDa (Hohn *et al.,* 1990). Barde and colleagues (1982) were the ones to discover the existence of this hormone and to show its neurotrophic properties in cultured sensory neurons. The molecular structure of BDNF is highly similar to that of the other neurotrophins and has remained homologues over species (*i.e.,* vertebrates, rodents, non-human primates, and humans) suggesting that BDNF has a long evolutionary history (Hallböök, 1999).

BDNF is encoded by a gene located on the short arm of chromosome 11 where it extends 70 Kb. The structure and functioning of the BDNF gene is complex as it consists of 9 exons and 11 promotor sites that all code for the same BDNF peptide variant (Liu *et al.,* 2005; Greenberg *et al.,* 2009). The transcription of BDNF is mainly initiated by neuronal activity and DNA methylation. Besides, there are a number of extrinsic stimuli that control the expression of BDNF (*a.o.,* steroids and inflammatory cytokines; see Reichardt 2006

for a review). Down-regulation of BDNF transcription occurs directly at the transcription site through antisense BDNF, which also is coded by the BDNF gene (Pruunsild *et al.,* 2007).

Two variants of BDNF peptides exist, a pro-form (pro-BDNF) and a mature form (mature BDNF, hereafter referred to as BDNF). After transcription, pro-BDFN is wrapped, packed in vesicles and transported into the Golgi-system. These vesicles can be spontaneously released, but unique for pro-BDNF is that its release also occurs in a stimulus dependent manner. This feature has been coined activity dependent secretion (Egan *et al.,* 2003). Activity dependent secretion is believed to be an important feature because it may reflect the nature of the nervous system to respond and to form synaptic modulations based on experiences. And this, as several authors have brought forward, may be a cellular manifestation of memory and learning (Katz and Schatz, 1996; Lu 2003). Pro-BDNF is secreted in the larger part of the central nervous system, including the hippocampus, the amygdala, and the cerebral cortex (Reichardt 2006). Intra-cellular, pro-BDNF is cleaved into mature BDNF by furin and pro-convertases proteases. In the extra-cellular space cleaving occurs by plasmin and matrix metalloprotease-9 (Lee *et al.,* 2001; Teixeira *et al.,* 2010). Pro-BDNF binds with high affinity to the p75 receptor and this has been associated with programmed cell death (*i.e.,* apoptosis; Boulle *et al.,* 2012; Park and Poo, 2013). So, pro-BDNF has biological significance beyond acting as a precursor for mature BDNF.

Mature BDNF is, just as pro-BDNF, expressed throughout the brain but highest concentrations can be found in the hippocampus and the frontal cortex, brain regions that are of crucial importance in the regulation of emotion, learning, and memory (Lindsay *et al*., 1994; Park and Poo, 2013). BDNF interacts with several receptor systems but has highest affinity with the Tyrosine kinase B receptor system (TrkB; Chao 2003). The binding of BDNF with TrkB results in intracellular phosphorylation and activation of intracellular signaling cascades that lead to the activation of so called pro-survival pathways, inactivation of pro-apoptotic signaling, and with neurogenesis (Ullrich and Schlessinger, 1990; Park and Poo, 2013). **Figure ↓** details the intra-cellular cascades that follow after the binding of BDNF with TrkB.

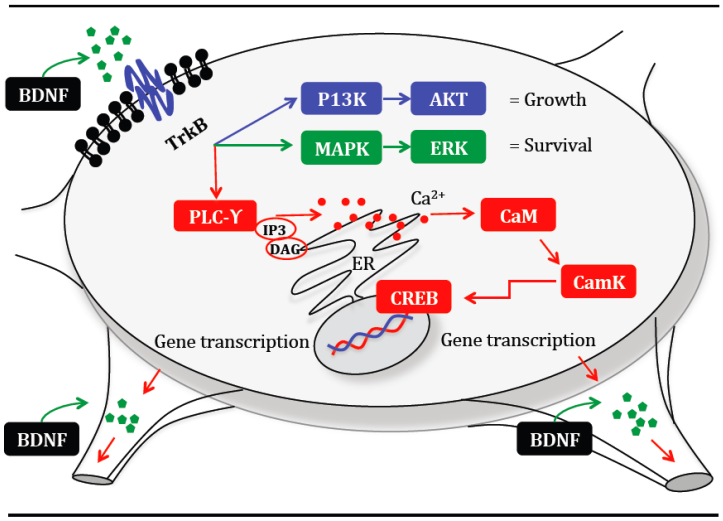

Figure 1. Overview of the signaling cascades that follow TrkB activation: (**I**) P13K/AKT (regulates translation initiation and neuronal survival); (**II**) MAPK (CREB phosphorilation); (**III**) PLC-γ (CREB phosphorilation).

Abbreviations:
BDNF, Brain-Derived Neurotrophic Factor
$Ca^{2+,}$ calcium
ER, Endoplasmatisch Reticulum
CREB, CAMP Response Element Binding
P13K, MAPK, Mitogen Activated Protein Kinase
Phosphatidylinositol 3 Kinase
PLC-, Phospholipase α C
TrkB, Tyrosine kinase receptor B

Adapted from: Green and Craddock (2005) and Nagahara and Tuszynski (2011).

It is useful to note that the mature BDNF variant also has some affinity with the p75 receptor system, the receptor that binds pro-BDNF with high affinity. Just as the coupling of pro-BDNF with p75, the coupling of mature BDNF with p75 is associated with apoptosis (Boulle *et al.,* 2012). So, depending on receptor type, BDNF may have seemingly opposing effects on neuronal cell survival and viability. This dissociation has been coined the Yin-Yang hypothesis of neurotrophic functioning (Lu *et al.,* 2005). Notwithstanding this, BDNF is regarded to be the key factor for initiating neurogenesis (the birth of new neurons), neuronal

survival (the selective survival from a larger set of neurons), and axonal outgrowth (Reichardt 2006). Furthermore, there is an association between BDNF activity and the prevention of apoptosis (*i.e.,* Kubo *et al.,* 1995; Li and Liu, 2010).

Some of BDNF's functions are dependent on developmental stage. It is for instance known that BDNF induces and supports the birth of new neurons early in development (Nagahara and Tuszynski, 2011) whereas in adulthood, BDNF is mostly associated with shaping the process of synaptic plasticity (Autry and Monteggia, 2012). An interesting perspective has begun to link the basal processes of apoptosis and neuronal plasticity to complex behavioral phenomenon, such as depression.

Major depressive disorder

With a lifetime prevalence of about 15 percent in community samples, depression is a common clinical disorder (Kessler *et al*., 2003). According to the World Health Organization (WHO 2004, 2008), depression is one of the leading causes of disease burden worldwide. Given its high prevalence and the large number of adverse personal and social consequences, depressive disorders bring enormous societal and economical costs (Greenberg *et al*., 1990; WHO 2008). Beyond this, the presence of depressive symptoms complicates the treatment of (other) chronic illnesses such as diabetes and it is, due to a related unhealthy life-style (*e.g.,* a poor diet, smoking) and relatively high rates of completed suicides, strongly associated with poor general health and morbidity (Harris and Barraclough, 1998; Harris *et al*., 2006). Adding to the adverse consequences of being depressed is that the illness frequently co-occurs (and/or shares overlapping symptoms) with personality pathology and substance use- and anxiety disorders (Kessler *et al.,* 2003, 2008; Kan *et al.,* 2005). **Box 1 ↓** lists the criteria that should be met to receive a diagnosis of a major depression.

BOX I. The diagnostic criteria for a depressive episode as they are stated in the DSM-IV TR (APA 1994)	
A. Depression may be diagnosed if five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (**I**) or (**II**)	
I	Depressed mood most of the day, nearly every day, as indicated by either subjective report (*e.g.,* feels sad or empty) or observation made by others. **Note:** In children and adolescents, this can be irritable mood
II	Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others)
III	Significant weight loss when not dieting or weight gain (*e.g.,* a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day
IV	Insomnia or hypersomnia nearly every day
V	Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)
VI	Fatigue or loss of energy nearly every day
VII	Feelings of worthlessness or excessive or inappropriate guilt nearly every day (not merely self-reproach or guilt about being sick)
IIX	Diminished ability to think or concentrate, or indecisiveness, nearly every day
IX	Recurrent thoughts of death, or a suicide attempt or a specific plan for committing suicide
B. The symptoms do not meet criteria for manic features in the presence or the past **C.** The symptoms cause clinically significant distress or impairment in important areas of functioning **D.** The symptoms are not due to the direct physiological effects of a substance or a general medical condition **E.** The symptoms are not accounted for by bereavement and persist for longer than 2 weeks **F.** The symptoms are not due to mood-incongruent delusions or hallucinations	

The diagnostic criteria for major depression are set forth in the Diagnostic and Statistical Manual version IV Text Revised (DSM-IV TR 1994) of the American Psychiatric Association (APA). The *core criteria* for a

diagnosis of depression are having *a depressed mood most of the day* and *a markedly diminished interest in almost all activities* persisting for longer than two weeks. A diagnosis of depression can be established if an individual meets at least five of nine pre-specified symptoms. The indication of whether a symptom is present is provided verbally by the patient or, in some instances, by the impression of the clinician. The symptoms that make up the illness major depression are descriptive and by no means are meant to provide an etiological model of the illness. This is largely so because the exact etiology of depression is unknown, although it is generally acknowledged that genetic predisposition and stress exposure play an immensely important role in it (APA 1994; Kendler 2012). One of the major aims of current psychiatric research is to go beyond mere description and move to a *hard medical model*, that is, a model that takes the etiological mechanisms of the illness into account (Kendler 2012).

The most common treatments for depression include pharmacological treatments (*e.g.,* antidepressants such as selective serotonin reuptake inhibitors), psychological therapies (*e.g.,* cognitive behavioral therapy), and many alternatives such as running therapy, electric shock treatment, sleep deprivation, and treatment with bright light. All these treatments are at best modestly effective in alleviating the symptoms of depression as only about half of the patients respond well to them (Mann 2005). This, the modest efficacy, is partly the result of an incomplete knowledge on the exact mechanisms on which treatment should focus. The relatively poor treatment outcome, together with the high prevalence, high burden and economical and social impact, make that the pathophysiology of depression needs to be understood much more clearly. Research on this topic hence deserves a high level of priority.

Current research into the etiology, treatment, and prevention of depression encompasses a great deal of approaches (cognitive, psychodynamic, interpersonal, genetic, genomic, proteomic and combinations). Since the 1980s however, theories crafted on biology- are dominant in providing answers with regard the forthcoming and the treatment of this illness. Although the pathophysiology of depression can be stratified over several biological domains (see Penninx *et al.* [2013] for a recent review), the two most prevailing paradigms are the *monoamine deficiency-* and the *neurotoxicity/stress hypothesis*. These hypotheses sketch a picture of altered brain function due to mostly monoamine dynamics, stress and genetic predispositions that together affect brain functioning in such a manner that depression may emerge. Such an approach may appear reductionistic in understanding a complex medical/psychological phenomenon as depression, yet they explain some key clinical observations with regard to it.

The *monoamine deficiency hypothesis*, in its original form put forth by Joseph Schildkraut in 1965, sketches a neuroanatomical basis for depression in the form of a deficiency in the expression of serotonin and noradrenaline in the brain (Hirschfeld 2000; Bunny and Davis, 1965). This hypothesis has been, without doubt, very useful. For instance, it served as benchmark for the discovery of antidepressant agents such as selective serotonin reuptake inhibitors that increase the availability of monoamines in the brain (Mann 2005). The theme of this hypothesis is elaborated in revised versions in which environmental events such as stress also are attributed to play an important role in illness initiation (*e.g.,* Henninger *et al.,* 1996; Caspi *et al.,* 2003).

Stress exposure forms the point of departure of the *neurotoxicity/stress hypothesis*. It suggests that stress is translated into biological process in which the illness origin of depression is embedded (Sapolsky 1990, 1996, de Kloet *et al.,* 1998). Indeed, stress exposure, particularly early in life, has a substantial association with depression onset (Sapolsky 1996; Charney 2004; Spinhoven *et al.,* 2010; Bogdan and Hariri, 2012). Stress is perceived in the brain where the hypothalamus reacts with the release of corticotrophin releasing hormone. Corticotrophin releasing hormone is projected on pituitary receptors that respond with the secretion of adrenocorticotrophin hormone. This, in turn stimulates the adrenal cortex to produce cortisol (Pariante and Miller, 2001). Cortisol expression has a range of short- and long-term effects on the

body (*e.g.,* sweating) and the brain (*e.g.,* high vigilance). Hyperactivation of this so-called hypothalamic-pituitary-adrenal axis is a consistent neurobiological abnormality in traumatized and depressed persons (Pariante and Miller, 2001) and not without effect, as structural brain damage may be a long-term cumulative effect of it (Videbech and Ravnkilde, 2004; van Harmelen *et al.,* 2010; Kang *et al.,* 2012).

Both the monoamine deficiency- and the neurotoxicity hypothesis received considerable support but they remain inadequate in some regards. For instance, the monoamine deficiency hypothesis has particular difficulties in explaining why it takes a number of weeks before the clinical efficacy of antidepressants kicks in and why in the larger part of persons (except maybe patients with a depressive disorder in the remission phase) a depletion of serotonin in the brain does not seem to produce depressive symptoms (Hirschfeld 2000; Lacasse and Leo, 2005). Furthermore, some clinically efficacious antidepressants (*e.g.,* tianeptine) actually are serotonin reuptake enhancers that, after ingestion, rapidly decrease the availability of serotonin in the synaptic cleft (Brink *et al.,* 2006). The neurotoxicity hypothesis also has some weaknesses. For instance, according to the theory, a down-regulation of corticotrophin releasing hormone should show antidepressant properties, but it does not (Mann 2005). A particular convolution for the theory further is that many persons experience depression without being exposed to (psychosocial) trauma or severe stress.

So, few would dispute that there is the urgency to move beyond these two models if one wishes to understand depression more fully. The *neurotrophin hypothesis* is believed to be such a step ahead. In the section that follows I will introduce the principles of this hypothesis and show (explain) why it has become a prevailing model of depression.

The neurotrophin hypothesis of depression

The first hints that led to the formulation of the *neurotrophin hypothesis of depression* came, as they often do, from studies on rodents**.** Based on the functions of BDNF, Smith and colleagues (1995) hypothesized that impairment in the expression of this hormone could lead to depressive-like behavior in rats. Indeed, these authors found this to be the case. Siuciak and colleagues (1996) in turn tested, also in rats, whether increasing BDNF expression in the brain could produce an antidepressant-like effect. Also these authors could confirm their hypothesis. Duman, Heninger and Nestler linked these findings to everyday clinical practice and to the monoamine deficiency- and the neurotoxicity hypothesis. This led, back in 1997, to the formulation of what has become known as the *neurotrophin hypothesis of depression*.

The rational for the neurotrophin hypothesis of depression is quite straightforward: BDNF expression, that is supposed to be shaped by genetic and environmental influences, can determine neuronal faith and viability and subsequently behavior, including depressive like behaviors, learning, and memory (Duman and Monteggia, 2006). The two basal predictions from this hypothesis are that depression results from a stress-induced decrease in BDNF expression and that antidepressants are efficacious because they normalize this (Duman and Monteggia, 2006; see **Figure 2 ↓**).

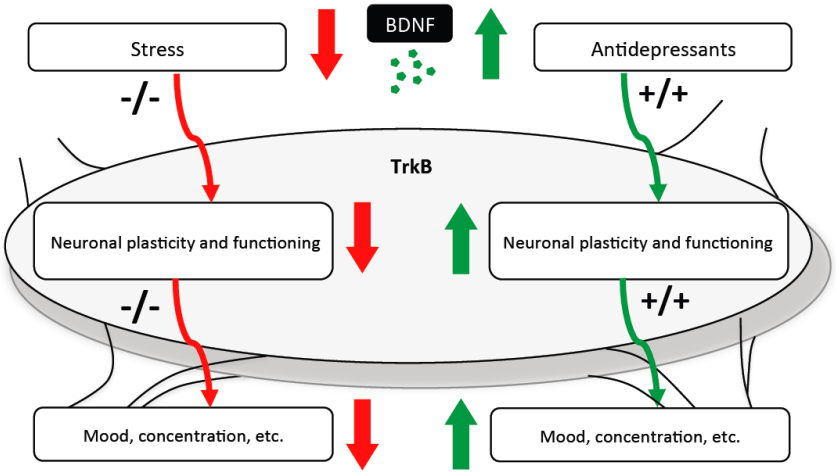

Figure 2. A schematic representation of the neurotrophin hypothesis

Abbreviations:
BDNF, Brain-Derived Neurotrophic Factor
TrkB, Tyrosine kinase B

Adapted from: Groves 2007

The neurotrophin hypothesis: pre-clinical evidence

The strongest evidence for involvement of the neurotrophic system in depression comes from animal studies. In a groundbreaking experiment, Malberg *et al.* (2000) showed that antidepressants increase adult hippocampal neurogenesis, a process that is under the direct influence of BDNF (Reichardt 2006). Similarly important was the finding by Santarelli *et al.* (2003) showing that if neurogenesis is blocked, the behavioral effects of antidepressants do not become evident. Here it should be noted that, only recently, compelling evidence has shown that substantial neurogenesis occurs throughout life in the adult human hippocampus (Spalding *et al.,* 2013). Together, these observations led to the belief that antidepressants are effective by virtue of a second messenger system (Dranovsky and Hen, 2006). This, the involvement of a second messenger system could in theory also explain the latency of weeks before the clinical efficacy of antidepressant treatment becomes evident (Rush *et al.,* 2006). Subsequent experiments could largely confirm the existence of such a system and it constituted, among others, out of BDNF expression (Zhao *et al.,* 2008). In a similar manner, BDNF is assumed to play a mediating role in stress-induced hippocampal atrophy (Hoshaw *et al.,* 2005; Kang *et al.,* 2012). In line with this are pre-clinical studies that have shown that stress reduces the expression of BDNF mRNA (*e.g.,* Prickaerts *et al.,* 2006) and clinical findings that show that cortisol expression is abnormally high in severely depressed persons (Anacker *et al.,* 2011).

Together these findings seem to suggest a common mechanism on depression initiation, progression, and treatment efficacy that goes beyond the neurotransmitter and receptor level. This mechanism is synaptic plasticity; *the ability of neurons to connect or disconnect as a function of use or disuse* (Park and Poo, 2013).

The neurotrophin hypothesis: clinical studies

A seminal advance for the neurotrophin hypothesis came from two studies on human subjects that were published in 2002 and 2003. Karege and colleagues (2002) were the first to show that serum BDNF concentrations are lower in depressed persons as compared to healthy control subjects. These authors further found that, within the group of depressed patients, serum BDNF concentrations were lower in the more severely depressed persons. A year later, Shimuzu *et al.* (2003) had a prime by showing an increase in serum BDNF concentrations in the course of antidepressant treatment. These findings that were thought of as being peripheral manifestations of the neurotrophic hypothesis greatly spurred the research activity on these topics. Replication attempts were subsequently published which served as input for two meta-analyses (Brunnoni *et al.* 2008; Sen *et al.* 2008). These

confirmed low serum BDNF concentrations in untreated depressed patients and normalization of this by antidepressant treatment.

In first stance, findings of abnormally low serum BDNF concentrations in depressed persons and normalization of this in the course of antidepressant treatment are seemingly important because they may help to parse out the pathophysiology of depression. In addition, a biological abnormality that is consistently reported and that is believed to be of relatively large effect-size may also be of value in clinical practice as a *biomarker* (*i.e.,* an objective [non-invasive] parameter that may aid in the classification of a diagnostic condition or in the assessment of treatment efficacy). As mentioned above, depressive disorders are diagnosed based on subjective verbal assessments without any referent to underlying pathophysiology (APA 1994). This may come with disadvantages in that such assessments may be inaccurate and colored by the state a patient is in or by the clinical impression of the patient by the therapist. A biomarker may in such instance be of help, as based on the score on it, a large and heterozygous group can be stratified in homogenous subgroups with as advantage that patients can be assigned to treatment options that best fit their needs (Kapur *et al.,* 2012).

An important question with regard to the above-presented findings relates to the sources of serum BDNF concentrations. A related question is whether peripheral differences in serum BDNF concentrations imply that there also are differences in the brain. Despite its name, BDNF is not solely derived from the brain. Other tissues, including several types of immune-, liver-, smooth muscle-, and vascular endothelial cells also serve as sources of BDNF (Cassiman *et al.,* 2001). Nonetheless, there are indications that BDNF measured in peripheral tissues reflects BDNF activity in the brain. These indications include pre-clinical findings that BDNF crosses the blood-brain barrier (Pan *et al.,* 1998) and positive correlations between peripheral and central BDNF concentrations (Klein *et al.,* 2010). The human data on this topic is, unfortunately, limited to only one study. In this particular study, blood was simultaneously derived from high up the jugular veins and from arterial veins, showing that BDNF levels were higher in blood that was derived from the internal jugular veins as compared to arterial blood (Dawood *et al.,* 2007). This indeed suggests that the source of BDNF in peripheral tissues can be found in the brain. For these reasons, it seems that neurotrophic functioning can be estimated from the periphery in a rather non-invasive manner. Corroborating this are human post-mortem studies that have indicated similar alternations in BDNF concentrations in the brains of persons who were depressed at the time of dying (*e.g.,* Thompson Ray *et al.,* 2011). Therefore, and given that for practical and ethical reasons data on central BDNF parameters is very hard to acquire, there is a great interest in peripheral BDNF measures in relation to depression and related phenotypes.

Besides the research on peripheral BDNF concentrations, other studies started to explore associations between variation on the gene that codes for BDNF and depression-related phenotypes. In the section that follows I will highlight some key studies that used this approach.

The BDNF gene, depression and related phenotypes

Of the 67,166 base pairs that make up the DNA sequence of the BDNF gene, one base pair clearly stands out with regard to the research attention that it received. This variant, known as BDNF val^{66}met (rs6265), refers to a locus where adenine and guanine vary resulting in a valine to methionine insertion at codon 66 (Egan *et al.,* 2003). This polymorphism comes in 3 variants: val homozygotes (val/val), heterozygotes (val/met), or met homozygotes (met/met; Petryshen *et al.,* 2010). In a groundbreaking study, Egan and colleagues (2003) showed, *in vitro*, that the met allele is linked to a reduced activity-dependent expression of BDNF in hippocampal neurons of rats. This finding has been replicated, *in vivo*, by Chen and colleagues (2006) and was further validated through animal studies using molecular

techniques such as knockout methods (Chourbaji *et al.,* 2004; Pandey *et al.,* 2008). Taking into mind the functions of BDNF, this finding of a functional variant on the BDNF gene sparked the interest of a lot of scientists, yielding a large output that I will summarize below.

In rodents it has been shown that the met variant at the val^{66}met locus is associated with aberrant dendritic spine formation in the hippocampus, which according to the neurotrophin and the neurotoxicity hypotheses constitutes a correlate or risk factor for depression (Spencer *et al.,* 2010). In line with this are recent findings by Bath and colleagues (2012) showing, in pre-clinical models, that the presence of a met allele is associated with greater anxiety- and depressive–like behavior. Some of these findings have been confirmed using data on human subjects. Highly relevant for the neurotrophin hypothesis were the findings of statistically significant associations between carrying a met allele and higher scores on depressive related traits (Montag *et al.,* 2008; Beevers *et al.,* 2009) and the DSM diagnosis of depression (Licinio *et al.,* 2009; Lavebratt *et al.,* 2010). Imaging studies have also provided evidence that is consistent with the neurotrophin hypothesis. Take for instance the findings by Pezawas *et al.* (2003) and Szeszsko *et al.* (2004) showing that carriers of a met allele have smaller hippocampal volumes (a phenotype associated with depression; MacQueen and Frodl, 2011; Spalding *et al.,* 2013) as compared to persons who are homozygous for the val allele. Besides, some studies have shown that carriers of a met allele do worse on tasks measuring cognitive performance (*e.g.,* Egan *et al.,* 2003). Finally, some studies have reported that the met variant is associated with lower peripheral BDNF concentrations (*e.g.,* Lang *et al.,* 2009; Ozan *et al.,* 2010). Based on the above (*i.e.,* functionality, associations with behavior and neuroanatomy), BDNF val^{66}met has become a very influential model to study neurotrophic functioning in a relatively non-invasive manner.

The neurotrophin hypothesis – not all that glitters is gold

As sketched above, the literature provides support for the notion that neurotrophic functioning may be at the heart of depression and related conditions such as anxiety. In addition, the literature largely is positive on (or at least gives ground for) the use of peripheral measures (notably serum BDNF concentrations) and certain genetic variants (notably BDNF val^{66}met) as parameters or proxies for neurotrophic functioning in the brain. For an overview of the breakthroughs in the research into the neurotrofin hypothesis I refer to the timeline in **Appendix I**.

Despite the marvel of findings that seem to have successfully related these *proxies* to behavior and processes that are associated with neurotrophic functioning, there however also are is uncertainty regarding the predictions of the neurotrophin hypothesis. Two main sources of this uncertainty are: (**I**) a lack of knowledge on the basic determinants of serum BDNF concentrations and (**II**) unanswered clinical questions regarding the neurotrophin hypothesis. In this thesis I will address these issues and so try to provide a more refined model of (peripheral) neurotrophic functioning in in depressive (and related) disorders.

Unexplored areas: the basal determinants of serum BDNF concentrations

One source of uncertainty regarding the predictions of the neurotrophin hypothesis is that next to nothing is known on the basal determinants/potential confounders of serum BDNF concentrations. We live in an associational world where phenomena cluster together. Because of this, characteristics (for instance behaviors or biochemical indices) may have a shared relation with a certain outcome without being genuinely associated to the outcome by itself (Smith and Ebrahimm, 2002). This has been coined as confounding; a phenomenon that complicates the interpretation of research findings and that easily can lead to erroneous inferences from the data and hitherto discordant *facts.* One solution in

minimizing the effects of confounding is to specify *determinants* because only then the opportunity arises to study independent associations. The first part of the prevailing thesis specifies the determinants of serum BDNF concentrations.

Unanswered clinical questions regarding the neurotrophin hypothesis

Another source of uncertainty is that some important clinical questions that are relevant in assessing the (construct and predictive) validity of the neurotrophin hypothesis remain unanswered. These questions include: (**I**) whether low BDNF concentrations persist beyond the clinical state of depression, (**II**) whether BDNF serum concentrations are related to the clinical characteristics of depression, such as its severity, (**III**) whether all types of antidepressants are equally associated with an upregulation of serum BDNF concentrations, and (**IV**) whether serum BDNF concentrations are also abnormally low in patients with an anxiety disorder. Here, I will try to answer these outstanding questions.

Furthermore, because the prominent role of stress and trauma exposure in the neurotrophin hypothesis and the etiology of depression, these factors need to be adequately explicated in BDNF related research, for instance by testing cross-term interaction effects among BDNF val^{66}met and trauma exposure on outcomes of interest (*e.g.,* hippocampal volume). To date, few studies have actually done this whilst it has been shown that such an approach can yield insight that otherwise would have remained hidden (see for instance Gatt *et al.,* 2009).

The purpose of this thesis

With the above in mind we set out to outline the basal determinants of serum BDNF concentrations and to resolve some important clinical questions regarding the neurotrophin hypothesis.

A notable add-on of the current work is that is that in order to achieve reliable effect-size estimates on associations of interest this thesis will use well-powered single studies and meta-analyses. This is important because findings related to the neurotrophin hypothesis are not consistently replicated. In fact, for basically all evidence in favor of the neurotrophin hypothesis, null and even opposite findings have been reported (*e.g.,* Elfving *et al.* [2012] for the finding that serum BDNF concentrations are low in depressed persons; Deuschle *et al.* [2013] for the finding that serum BDNF concentrations are up-regulated in the course of antidepressant treatment; Terracciano *et al.* [2011] and Gerritsen *et al.* [2011] for the associations between val^{66}met and serum BDNF concentrations and hippocampal volumes respectively). These discrepancies may be sample-related and for instance due to between-study differences in patient recruitment, patient status, or antidepressant dosages. Other mundane reasons are methodological in nature and notably include the use of an underpowered study design (Button *et al.,* 2013; Murad and Montori, 2013). Nothwitstanding the exact reason, the current thesis will provide reliable effect-size estimates through the use of well-powered single studies and meta-analytical techniques to (dis)confirm the rigour of its own findings.

Through all this I hope to facilitate ongoing research into neurotrophic functioning in depression (and related illnesses). This, to my belief, is of eminent importance because it may add to the understanding of the pathophysiology of depression, a common and debilitating illness that needs to be better understood.

Outline of this thesis

The foregoing text broadly provided the theoretical background of this thesis. The chapters that follow go beyond description and are empirical in nature. The first aim of this thesis, on delineating the basic determinants of serum BDNF concentrations, is described in **chapter 2** and **3**. **Chapters 4** till **9** are

devoted to the second aim of this thesis, that is to answer important clinical questions regarding the neurotrophin hypothesis

Chapter 2 provides a description of the basic determinants (sampling-, socio-demographic-, and life-style characteristics) of serum BDNF concentrations. **Chapter 3** describes seasonal entrainment of serum BDNF concentrations. **Chapter 4** (a single study design) and **chapter 5** (a meta-analysis) describe the author's efforts to advance the understanding of the associations between serum BDNF concentrations and the illness major depression, its characteristics (*e.g.,* the course illness), and the use of antidepressants. **Chapter 6** evaluates whether abnormalities in serum BDNF concentrations are evident in persons diagnosed with an anxiety disorder. In **chapter 7** I report a study on the effect of BDNF val^{66}met on serum BDNF concentrations and whether this presumed effect is conditional upon exposure to childhood trauma. In **chapter 8** and **chapter 9** we extend our outcome measures beyond serum BDNF concentrations to the volume and the functioning of the hippocampus and to cognitive performance. Specifically, in **chapter 8** we ascertain whether variation at the BDNF val^{66}met locus, in interaction with stress exposure in child- and adulthood, is consistently associated with hippocampal volume and functioning and with cognitive performance. **Chapter 9** contains a systematic review and meta-analysis on the association between BDNF val^{66}met and hippocampal volume. Finally, in **chapter 10**, I will aggregate and discuss our findings, address a vast array of pitfalls and limitations of our work and acknowledge objections to the way in which I interpreted the data. Finally, the possible implications of the work herein are reviewed and the main open questions are stipulated.

CHAPTER 2

Determinants of serum BDNF concentrations

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SIGNIFICANCE: In this study we sketch the basic determinants of serum BDNF concentrations. Herewith we offer an improved base to understand inter-individual differences in serum BDNF concentrations and knowledge that is essential in preventing erroneous inferences from data.

ABSTRACT

Brain-Derived Neurotrophic Factor (BDNF) belongs to the neurotrophin family of growth factors and affects the survival and plasticity of neurons in the adult central nervous system. The high correlation between cortical and serum BDNF concentrations has led to many human studies on BDNF concentrations in various populations, however knowledge about determinants that influence BDNF is lacking. To gain insight into the factors that influence BDNF concentrations in humans, we measured in 1,168 people aged 18 through 65, free of antidepressants and current psychiatric disease four categories of determinants (sampling, socio-demographics, lifestyle indicators and diseases) were measured as well as serum BDNF concentrations. We used univariable analyses and multivariable linear regression analyses in particular to determine which of the possible determinants significantly influenced serum BDNF concentrations. Our final multivariable regression analysis revealed that a non-fasting state of blood draw, later measurement, longer sample storage and being a binge drinker all were associated with attenuated BDNF concentrations. This was in contrast to smoking and living in an urban area, which was associated with increased BDNF concentrations. Moreover we found that older subjects also had higher BDNF concentrations, but this only applied to women. Future studies on serum BDNF concentrations in humans should correct for the time of blood withdrawal, duration of serum storage, urbanicity, age, gender, smoking status and food and alcohol intake.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) belongs to the neurotrophin family of growth factors and affects the survival and synaptic plasticity of neurons in the adult nervous system (Mossner *et al*., 2007). BDNF binds with the TrkB tyrosine kinase receptor (Chao, 2003), which results in intracellular phosphorylation and activation of signaling cascades that lead to activation of pro-survival pathways (Ullrich and Schlessinger, 1990). BDNF is expressed throughout the central nervous system (Binder and Scharfman, 2004) and significant concentrations are also found in peripheral blood (Sen *et al*., 2008). BDNF crosses the blood-brain barrier by a saturable transport system (Pan *et al*., 1998). In animal models, cortical BDNF concentrations are highly correlated with peripheral serum BDNF concentrations (Sartorius *et al*., 2009), but evidence on the contrary has been reported as well (Elfving *et al*., 2010). Inconsistencies might be explained by the fact that BDNF-expression and its TrkB receptor is not specific for neuronal cells, but can also be found in endothelial cells in peripheral tissues (Esteban *et al*., 1995; Hiltunen *et al*., 1996; Donovan *et al*., 2000). The supposed importance of neuroplasticity in both the etiology and recovery of psychiatric disorders, has led to many studies linking serum BDNF concentrations to a wide variety of psychiatric and neurodegenerative diseases (Nakazato *et al*., 2003; Azoulay *et al*., 2005; Yasutake *et al*., 2006; Ciammola *et al*., 2007; Ikeda *et al*., 2008). For proper interpretation of serum BDNF concentrations in humans, however, knowledge about determinants of serum BDNF concentrations is essential for adequate control of confounding factors.

As touched above, BDNF is not only considered to be a neurotrophin, but also an immunotrophin, epitheliotrophin and metabotrophin (Chaldakov *et al*., 2007). As of yet somatic conditions that have been correlated to BDNF mainly include cardiovascular disease or cardiovascular risk factors or disease (Ejiri *et al*., 2005; Fujinami *et al*., 2008; Hristova and Aloe, 2006; Suwa *et al*., 2006; Geroldi *et al*., 2006). For example, patients with acute coronary syndrome as well as patients with diabetes mellitus do have lower plasma concentrations of BDNF (Manni *et al*., 2005; Fujinami *et al*., 2008). However, spurious associations may be explained due to (unknown) confounding factors, since health indicators like alcohol use, smoking and physical exercise have also been linked to BDNF (Chan *et al*., 2008; Tang *et al*., 2008; Umene-Nakano *et al*., 2009). Even more basic determinants, like sampling characteristics have not specifically been examined regarding their association with serum BDNF concentrations. Although most human studies control for gender and age effects, reported effects of gender and age are contradictory. These mixed results may be explained by small sample sizes ranging from as low as 10 (Marano *et al*., 2007) through a maximum of 465 participants (Ziegenhorn *et al*., 2007), as well as failure to control for potentially confounding factors (Aydemir *et al*., 2007). Finally, possible effects of sampling characteristics on serum BDNF concentrations have hardly been examined. The few studies available, however, do suggest a decrease of BDNF concentrations after longterm storage (Trajkovska *et al*., 2007) and a diurnal variation within individuals (Piccinni *et al*., 2008). Considering the broad interest in BDNF and the many factors associated with serum BDNF concentrations, it is surprising that no studies have been conducted to determine the effects of potential determinants of serum BDNF concentrations. Therefore, we examined the determinants (sampling characteristics, socio-demographic variables, lifestyle indicators and [chronic] diseases) of serum BDNF in a large cohort of people without current psychiatric diseases.

METHODS

Data are from the baseline measurement of the Netherlands Study of Depression and Anxiety (NESDA). NESDA is a multisite naturalistic cohort study aimed to describe the 8-year course and consequences of depressive and anxiety disorders and to integrate biological and research paradigms

within an epidemiological approach. Recruitment took place in the general population, in general practices and in mental health organizations. The baseline sample consists of 2,981 participants aged 18 through 65 years of which 2,625 were selected on the basis of a current or life-time history of depression and/or anxiety disorder, belonging to a high-risk group because of a family history or sub-threshold depressive or anxiety symptoms, and 356 healthy controls. Patients with a primary psychiatric disorder not subject of NESDA, such as psychotic disorder, obsessive-compulsive disorder, bipolar disorder, or severe addiction were excluded as well as those not being fluent in Dutch. The Medical Ethics Commission of the participating institutes approved of this study and all respondents provided written informed consent.

All respondents received a structured diagnostic interview using the Composite International Diagnostic Interview (CIDI version 2.1) in order to assess current and lifetime DSM-IV diagnoses. The 4 hour baseline assessment further included written questionnaires, interviews, a medical examination, and collection of blood and saliva samples, in order to gather extensive information about key (mental) health outcomes and demographic and clinical determinants. All measurements and interviews took place were carried out by our specially trained staff according to a previously determined protocol. For a detailed description of the objectives and methods of NESDA, see Penninx *et al*. (2008). Based on our previous study (Molendijk *et al*., 2010) showing that BDNF was associated with both current psychopathology and antidepressant usage, but not with trait psychopathology or sub-threshold symptoms, we excluded only participants with a current DSM-IV diagnosis of a depressive disorder or anxiety disorder in the last 6 months (*n* = 1,688) and those who were using any kind of antidepressant medication (*n* = 736). This resulted in a study population of 1,198 participants. Of these, 30 did not have serum BDNF assessment and 3 samples were below the detection limit. These persons therefore were excluded, leaving a final sample of 1,165 for the present study.

BDNF assessment

Blood was collected (between 06:20 and 12:30 h) after an overnight fast and immediately transferred to one of the five local laboratory-sites to start processing within 1 h. Serum samples for BDNF assessment were stored at -85 C˚. After concluding the baseline assessment, serum samples were sent (ranging from 22 to 60 months after withdrawal) to the Department of Psychiatry and Neuropsychology in Maastricht (The Netherlands) for BDNF measurements. Serum BDNF protein concentrations were measured within 3 months after their receipt using the Emax Immuno Assay system from Promega according to the manufacturer's protocol. The undiluted serum was acid treated, which in a dilution-dependent way reliably increased the detectable BDNF. Subsequently, serum samples were diluted 100 times and stored again at -85 C˚ for BDNF assay the next day. After dilution, the BDNF concentrations were well within the range of the standard curve. The assay sensitivity threshold was ascertained at 1.56 ng/ml reflecting the minimum level of BDNF in the serum that could be reliably determined. Three samples were below this threshold and deleted for all subsequent analyses (see also above). In our pilot study we had found that BDNF concentrations of acid-treated samples with subsequent dilution the day preceding the BDNF assay did not differ from the concentrations obtained in samples following acid treatment only on the preceding day, or from those derived after acid treatment conducted the day of the BDNF measurement. The serum samples used in our pilot study were stored samples of six individuals who did not participate in the present study. The samples' coefficients of variance ranged from 2.9% to 8.1%. To gauge the intra-assay variance for the present study, we analyzed two of our current samples on two different plates on the same day. The resultant coefficients of variance of 0.1% and 3.1% were both well below the maximum

intra-assay variance of 8.8% as specified by the manufacturer. Greiner Bio-One high affinity 96-well plates were used and the resulting absorbance was read in duplicate using a Biorad Benchmark microplate reader at 450 nm.

Potential determinants of BDNF

Sampling variables

The sampling variables that were tested consisted of time of blood withdrawal (minutes after 6.00 a.m.), the number of minutes a sample was kept in a cool box before being processed in the local laboratory, the duration of sample storage at -85 C˚ (days) and finally non-adherence to the pretest fasting protocol.

Socio-demographic variables

The socio-demographic variables that were tested consisted of gender (male/female), age (years), urbanicity of living environment of participant (urban/not urban) and years of education. An urban environment was defined according to the classification of the Dutch office for statistics (CBS) and dichotomized in more or less than 1000 addresses per square kilometer. As a previous study of our group suggested the presence of an age effect only in women (Bus *et al*., unpublished observations), we were also interested in the interaction between age and gender a priori.

Health indicators

The health indicators that were tested in this study included presence of metabolic syndrome, smoking, physical activity (met-minutes) and drinking (abstainer, mild, moderate, excessive) and body mass index (BMI). Metabolic syndrome was defined according to the updated Adult Treatment Panel III (ATP III) guidelines of the US *national* cholesterol education program (Grundy *et al*., 2005) in which metabolic syndrome is considered present if a participant meets at least three of the following criteria: (1) elevated waist circumference (men > 102 cm; women > 88 cm); (2) elevated triglycerides (> 150 mg/dl); (3) reduced HDL cholesterol (men < 40 mg/dl; women < 50 mg/dl); (4) elevated blood pressure (> 130/85 mm Hg or use of medication for hypertension); or (5) elevated fasting glucose (> 5.6 mmol/l or use of medication for hyperglycemia). As a measure for physical activity we used Metabolic Equivalent of Task (MET)-minutes. A MET-minute is a ratio of the amount of energy expenditure during an activity to the expenditure at rest, which was calculated on the basis of the international physical activity questionnaire (Craig *et al*., 2003). Smoking was dichotomized into current smokers versus non-smokers. In addition to current smoking, we also collected information on smoking status in the past (*i.e.,* age of starting, age of quitting) and the average number of cigarettes smoked a day. The number of package years was calculated with one package year defined as smoking 25 cigarettes a day for the period of 1 year (= 9,125 cigarettes). The Fagerstrom questionnaire was used to measure the severity of nicotine dependence (Heatherton *et al*., 1991). Alcohol use was evaluated by creating four groups: abstainers, mild drinkers (drinking less than 7 units a week), moderate drinkers (drinking 8-13 units a week) and excessive (drinking ≥ 14 units a week). Alcohol categories were entered as dummy variables. In addition the AUDIT was used to measure alcohol dependence (Babor *et al*., 1989). BMI was calculated by dividing weight by the squared height and entered as a continuous variable.

Disease indicators

Based on previous associations with serum BDNF concentrations in humans, we evaluated the presence of Chronic Non- Specific Lung Disease (CNSLD; including asthma, chronic bronchitis and

chronic emphysema) and coronary artery disease (Fujinami *et al*., 2008). The latter was defined as self-reported vascular events due to atherosclerotic disease (*i.e.,* stroke, angina pectoris, myocardial infarction or a history of percutaneous transluminal coronary angioplasty or coronary artery bypass grafting). Stroke, myocardial infarction and angina pectoris were only considered present if supported by appropriately prescribed medication use.

Statistical analysis

Serum BDNF concentrations were normally distributed, with the exception of 12 (0.5%) positive outliers (*i.e.,* level higher than 3 standard deviations (SD) above mean). Outliers were handled by trimming all serum BDNF concentrations above 3 *SD*s of the mean to the 3 *SD*s value. Univariable analyses were carried out using linear regression. We performed multiple linear regression analyses to assess independent determinants of BDNF. To facilitate the interpretation of the age-gender interaction, rather than taking absolute ages, we calculated and included the deviation from the mean, so that the participants' ages were centered round the sample's mean age. We generated multivariable models within each domain first by entering sampling characteristics, socio-demographic characteristics, health indicators and disease variables in four separate models. Subsequently, the independent predictors from all domain-specific models with *P*-values less than .15 were fitted into a final multivariate model. No collinearity or heteroscedasticity problems emerged. All tests were two-sided. A *P*-value of 0.05 was considered statistically significant. All analyses were carried out using SPSS 16.0 (SPSS, Chicago, IL, USA).

RESULTS

Sample characteristics

The 1,165 participants had a mean age of 42.5 years (SD = 14.1) and 65.0% (757) were female. The mean BDNF level was 8.98 ng/ml (SD 3.1 mg/ml) with a range from 1.56 ng/ml through 18.50 ng/ml. **Table 1 ↓** presents all further characteristics as well as the univariable associations of these characteristics with serum BDNF concentrations. As shown in **Table 1 ↓**, a lower serum BDNF level was associated with non-adherence to pretest fasting protocol (ß = 0.07; *P* = .01), sampling at a later day-time (ß = 0.07; *P* = .02), a longer time of storage (ß = 0.09; *P* = .002), high alcohol intake (excessive drinkers versus others (ß = 0.073; *P* = .020) and finally increased physical activity (ß = 0.06; *P* = .03). A higher BDNF serum level was found in older patients (ß = 0.21; *P* < .001), a higher degree of urbanicity (ß = 0.15; *P* < .001), current smokers (ß = 0.07; *P* = .01) and in patients suffering from the metabolic syndrome (ß = 0.06; *P* = .03) or coronary artery disease (ß = 0.08; *P* = .005).

Multivariable analyses

From the four models, categorized by variable type, we identified eleven significant determinants. Participants that had eaten prior to blood withdrawal had significantly lower serum BDNF concentrations (ß =0.07; *P* = .02). Sampling later during the morning resulted was associated with lower serum BDNF level (ß = 0.07; *P* = .01) as well as longer sample storage (ß = 0.08; *P* = .005). Serum BDNF concentrations in women significantly increased with age (ß = 0.23; *P* < .001), but also in men (ß = 0.10; *P* = .03). The age change was significantly different in women compared to men, as was indicated by the significant interaction between gender and age (ß = 0.08; *P* = .02).

Subjects who were living in a more urbanized area had higher BDNF concentrations (ß = 0.09; *P* = .002). Excessive drinkers had significantly lower serum BDNF concentrations compared with subject with more restraint drinking habits, as was indicated by the significant dummy variable (ß = 0.08; *P* =

.009). Current smokers had a significantly higher BDNF level compared to non-smokers (ß = 0.09; *P* = .003). Subjects with more physical activity had lower serum BDNF concentrations (ß = 0.06; *P* = .04).

Patients with cardiovascular disease had higher serum BDNF concentrations (ß = 0.08; *P* = .005). All these variables were entered in the final model. The presence of metabolic syndrome did not reach statistical significance (ß = 0.05; *P* = .12), but was entered into our subsequent model as well on the basis of a statistical significance at a *P* < .15 level. Our final multivariate regression analysis revealed that, when accounting for all potential determinants at once, having eaten prior to blood withdrawal (ß = 0.07; *P* = .01), measurement later on the day (ß = 0.06; *P* = .02), longer sample storage (ß = 0.08; *P* = .004), and being an excessive drinker (ß = 0.06; *P* = .03) all were associated with attenuated serum BDNF concentrations. This was in contrast to smoking (ß = 0.10; *P* = .001) and living in an urban area (ß = 0.11; *P* < .001), which resulted in increased BDNF concentrations. Moreover we found that older subjects also had higher BDNF concentrations, but this was only true for women (ß = 0.23; *P* < .001). The age change did not reach statistical significance for men (ß = 0.06; *P* = .26) and was significantly different from age change in women (ß = 0.10; *P* = .007)(see **Table 2 ↓**).

Table 1. Sample characteristics (*N* = 1,165) and univariable associations with serum BDNF concentrations

	Mean (*SD*) value or % (*n*)	Linear regression	
		ß	*P*-value
Sampling variables			
% fasting on withdrawal day	95.3 (*n* = 1,110)	-0.07	0.01
Time of sampling (mean minutes past 0600 h)	168 (*SD* = 20)	-0.07	0.02
Time in coolbox (mean minutes)	60 (*SD* = 43)	0.03	0.35
Duration of sample storage (#days)	1286 (*SD* = 228)	-0.09	< 0.01
Sociodemographics			
% female	65.0 (*n* = 757)	0.02	0.42
Age (mean in years)	42.5 (*SD* = 14.1)	0.21	< 0.01
% living in urban area	85.9 (*n* = 1,001)	0.15	< 0.01
Education level (mean in years)	12.7 (*SD* = 3.1)	0.05	0.12
Lifestyle indicators			
% currently smoking	30.3 (*n* = 353)	0.07	0.01
% with metabolic syndrome	19.1 (*n* = 222)	0.06	0.03
Physical activity (#mean MET-minutes)	3708 (*SD* = 3108)	-0.06	0.03
% abstinent of alcohol	44.8 (*n* = 522)	Ref.	
% mild drinker; <1U/day	34.2 (*n* = 399)	-0.07	0.82
% moderate drinkers; 1-2U/day	5.9 (*n* = 69)	-0.02	0.57
% excessive drinkers; >2U/day	3.6 (*n* = 42)	-0.07	0.02
BMI (height/weight^2)	25.3 (*SD* = 4.5)	0.04	0.22
Disease			
% with coronary artery disease	5.3 (*n* = 62)	0.08	< 0.01
% with CNSLD	10.0 (*n* = 117)	< 0.01	0.98

Abbrevations: SD = standard deviation; U = unit; BMI = body mass index; CNSLD = chronic non-specific lung disease; MET = metabolic equivalent of task

Post-hoc analyses

Significant associations in the univariable or multivariable analysis were analyzed in more depth by post hoc analyses using other definitions for the variable or including potentially explanatory variables. For example, in order to explain gender differences, we examined the use of oral contraception.

Gender differences

In order to further explore the gender differences, we assessed the influence of menopausal status and contraceptive pill use in women on BDNF concentrations. Since age and menopausal status are interwoven variables, we selected women in a narrow age range of 48-52 years of age. Since pre- and postmenopausal women were evenly dispersed (premenopausal *n* = 41; postmenopausal *n* = 40) in this age range, we minimized the confounding influence of age. The age variable was entered in a multiple regression together with a dichotomous menopausal status variable and the interaction between these two variables. We corrected for variables previously found to be of influence. Premenopausal women showed a significant increase in serum BDNF concentrations with age (ß = 0.46; *P* = .02) and differed significantly (*P* = .03) from post-menopausal women who showed a non-significant decrease (ß = -0.19; *P* = .43). Pre- and postmenopausal women's BDNF serum concentrations did not significantly differ (ß = 0.12; *P* = .43) at the age of 50.1 years (mean age of this sample). To test whether contraceptive pill use influenced BDNF concentrations we selected all premenopausal women and entered the variable: yes versus no contraceptive pill use) in our final model. This, however, revealed a non-significant result for contraceptive pill use (ß = 0.03; *P* = .53).

Table 2. Multiple linear regression analyses (per block and overall analyses)

	Multivariate per block				Final multivariate model			
Variables in four blocks	*B*	*SE*	β	*P*-value	*B*	*SE*	B	*P*-value
Sampling variables								
Fasting on withdrawal day (0 = no, 1=yes)	-10.03	0.443	-0.07	0.02	-10.01	0.43	-0.07	0.02
Time of sampling (minutes after 0600h)	-0.01	< 0.01	-0.07	0.01	-0.01	< 0.01	-0.07	0.02
Time in coolbox (minutes)	< 0.01	< 0.01	0.01	0.75	-	-	-	-
Duration of sample storage (days)	-0.001	< 0.01	-0.08	< 0.01	< -0.01	< 0.01	-0.08	< 0.01
Sociodemographics								
Gender (0 = female, 1 = male)	0.10	0.19	0.02	0.58	0.06	0.19	0.01	0.74
Age (years deviating from mean age)	0.05	< 0.01	0.23	< 0.01	0.05	< 0.01	0.23	< 0.01
Interaction age times gender	-0.03	0.01	-0.08	0.02	-0.04	0.01	-0.10	< 0.01
Living in urban area (0 = no, 1=yes)	-0.84	0.27	0.09	< 0.01	-0.98	0.26	0.11	< 0.01
Education level (years)	0.04	0.03	0.04	0.19	-	-	-	-
Lifestyle indicators								
Current smoking (0 = no, 1 = yes)	0.599	0.20	0.09	< 0.01	0.66	0.19	0.10	< 0.01
Metabolic syndrome (*n*)	0.419	0.27	0.05	0.12	< 0.01	0.24	< 0.01	0.99
Physical activity (MET-minutes)	< -0.01	< 0.01	-0.06	0.05	< -0.01	< 0.01	-0.05	0.07
Alcohol drinking dummy (0 vs > 2U/day)	-10.31	0.50	-0.08	< 0.01	-10.03	0.49	-0.06	0.04
Alcohol drinking dummy (0 vs 1-2U/day)	-0.58	0.40	-0.04	0.15	-0.477	0.40	-0.04	0.23
Alcohol drinking dummy (0 vs < 1U/day)	-0.21	0.21	-0.03	0.31	0.05	0.20	< 0.01	0.80
Body mass index (weight/height^2)	< 0.01	0.02	0.01	0.77	-	-	-	-
Disease								
Coronary artery disease (0 = no, 1 = yes)	10.13	0.40	0.082	< 0.01	0.54	0.41	0.04	0.18
CNSLD (0 = no, 1 = yes)	< 0.01	0.30	< 0.01	0.99	-	-	-	-

Abbrevations: SD = standard deviation; *SE* = standard error; U = unit; CNSLD = chronic non-specific lung disease; MET = metabolic equivalent of task

Smoking

We created dummy variables for former and current smokers and subjects who have never smoked and entered these into our final model, thus correcting for potentially confounding factors. This

revealed that current smokers had significantly higher BDNF concentrations compared to those who have quit smoking (ß = 0.15; *P* < .001). However, no significant difference was found between current smokers and those who have never smoked (ß = 0.05; *P* = .11). Furthermore, no effect on serum BDNF concentrations was found of package years, actual number of cigarettes a day among smokers, as well as the severity of nicotine dependence as assessed with the Fagerstrom questionnaire (data not shown).

Alcohol use

In order to check whether the effect of alcohol intake on serum BDNF concentrations could be explained by the degree of alcohol dependence, we replaced the alcohol variables in our final model with the sum score of the AUDIT. No significant effect was found of the total AUDIT score on serum BDNF concentrations (ß = 0.05; *P* = .10).

Cardiovascular disease

Since the metabolic syndrome and cardiovascular disease were associated with serum BDNF concentrations in the univariable analysis, we further investigated this by repeating our multivariable analysis in a stepwise manner. We found that significance for both metabolic syndrome and cardiovascular disease was lost after the introduction of age as a variable. Moreover we investigated the individual components of metabolic syndrome, by adding these to our final regression model as described above. Only hyper-triglyceridemia significantly contributed to the variation in serum BDNF level (ß = 0.07; *P* = .018). The four other individual criteria did not reach statistical significance: low HDL cholesterol (ß = 0.01; *P* = .91), abdominal obesity (ß = 0.02; *P* = .44), hypertension ß = 0.01; *P* = .87), hyperglycemia (ß = 0.04; *P* = .17).

DISCUSSION

Main findings

Within a large cohort of people free from psychiatric disorders, we identified eight independent determinants of serum BDNF concentrations: time of blood withdrawal, time of storage, food intake before sampling, urbanicity, age, gender, smoking status and drinking behavior. Below we will discuss these determinants in more depth.

Sampling variables

Three sampling variables had an independent effect on serum BDNF concentrations in our study. First, although the overall decline was small, BDNF concentrations were significantly lower after long-term storage over a mean period of about 3.5 years. This is in line with previous results showing that serum BDNF had significantly decreased after 5 years of storage, but not yet after 12 months (Trajkovska *et al*., 2007). In our study, however, this decline of serum BDNF was far less pronounced. Most likely, this can be explained by storage temperature, which was much lower in our study (*i.e.,* -85 C˚ compared to -20 C˚). It is important to know, especially for epidemiological studies taking blood samples outside the hospital that the time kept in a cool box before initial processing does not affect BDNF serum concentrations. Secondly, we found attenuated serum BDNF concentrations when blood was drawn later in the morning. Diurnal variation has been reported for plasma BDNF concentrations, but not for serum BDNF concentrations (Piccinni *et al*., 2008). Acknowledging the limited time-interval in which serum BDNF concentrations were measured in our study, a much larger diurnal variation in BDNF can be expected. This should be explored in more detail in subsequent studies. Finally, to our knowledge,

we are the first to show that food intake prior to sampling results in lower serum BDNF concentrations. Although we cannot exclude that these results were caused by selection bias, it does plead for taking this into consideration in future research.

Socio-demographic variables

In contrast with men, in whom change in BDNF concentrations by age is far less pronounced, in our sample of people aged between 18 and 65 years we found increasing serum BDNF concentrations in women with advancing age. In a previous study of our group among community-dwelling older people aged 50 through 72 years we found constant BDNF serum concentrations in men, whereas in women an age-related decrease was found. At a first glance, these results seem puzzling, but the difference between men and women, as well as the opposite age related effects in younger and older women, might be explained by gender-hormone differences between men and women. Post-hoc analyses found a significant interaction of age and menopausal state in women, with an age-related increase of serum BDNF concentrations in premenopausal women and an age-related decrease in postmenopausal women. As estrogen concentrations are significantly associated with BDNF concentrations (Monteleone *et al*., 2007), the postmenopausal drop in estrogen concentrations could possibly result in decreasing BDNF serum concentrations. These results thus suggest that in women serum BDNF concentrations peak at the climacteric age. However, since age and menopausal status are inextricably linked, it remains difficult to draw any conclusions in this cross-sectional approach, hence warranting future longitudinal research. Furthermore, participants living in an urban environment had significantly higher serum BDNF concentrations compared to their rural counterparts. The effect of urbanicity might be hypothesized to be an indirect effect mediated by different factors. On the one hand, one may expect decreased BDNF concentrations due to a higher exposure to stress factors (Godfrey and Julien, 2005) and higher frequency of psychopathology (Peen *et al.,* 2009). On the other hand, one may expect increased BDNF concentrations due to a higher environmental enrichment, which has shown to induce BDNF gene expression (Kuzumaki *et al.,* 2010). As we excluded participants with current psychopathology (and associated the highest stress concentrations), this latter effect might have outweighed the effect of stress and psychopathology.

Health indicators

Only two health indicators were associated with serum BDNF concentrations. First, current smoking was associated with higher BDNF concentrations. Interestingly, an opposite effect has been reported on plasma BDNF concentrations in two previous studies (Bhang *et al*., 2010). Assuming that previous results are not chance findings due to low patient numbers, these opposite effects might be explained by dysfunctioning platelets in smokers resulting in impaired BDNF-release from platelets, which would affect plasma but not serum concentrations of BDNF (Nowak *et al*., 1987). Despite the clear effect of current smoking, we neither detected a dose-response effect measured by package years (in smokers and ex-smokers) nor a dose-response effect measured by number of cigarettes a day (in current smokers). Different hypotheses seem valid. First these findings might indicate a direct toxic effect of smoking, as in animal research a direct relationship between nicotine and BDNF expression in the brain has been reported (Kim *et al*., 2007).

Secondly, the association might be caused by an underlying third factor related to serum BDNF concentrations as well as smoking habits (vulnerability for starting smoking or being able to quit when started), as preliminary evidence has found an effect of val^{66}met polymorphism of the BDNF gene on smoking habits (Lang *et al*., 2007), although a replication was negative (Montag *et al*., 2008).

Moreover, in our sample we could not find an association between the degree of nicotine dependence and serum BDNF concentrations and previous studies (performed in plasma BDNF) showed that subjects had BDNF concentrations similar to non-smokers after 12 weeks of smoking cessation (Bhang *et al*., 2010). This evidence points towards a direct involvement of the inhalation of smoke in influencing BDNF concentrations. High alcohol intake, but not the severity of alcohol dependence, was associated with lower BDNF serum concentrations in our sample. BDNF has been hypothesized to be implicated in ethanol-induced neuro-degeneration in the adult brain, but to date, the role of BDNF in alcohol use disorders is still a matter of debate (Davis 2008). The only study of serum BDNF concentrations in patients with alcohol dependence found only diminished BDNF concentrations in case of co-morbid depression, but not in patients with alcohol dependence without co-morbid depression (Umene-Nakano *et al*., 2009). This picture becomes even more complicated acknowledging the possible role of the val^{66}met polymorphism of the BDNF gene in relapse of alcohol use disorders (Wojnar *et al*., 2009). More research on the role of BDNF in alcohol use disorders is clearly warranted. Thus far, studies investigating BDNF outside the area of alcohol use disorder should exclude people who use more than 14 standardized units of alcohol a week. The small inverse relationship between physical activity and BDNF serum concentrations showed a tendency toward statistical significance. Previous studies reported increased serum BDNF concentrations directly after exercise (Tang *et al*., 2008), but decreased basic BDNF concentrations at rest in physically more active humans (Currie *et al*., 2009).

Chronic disease

Although there is evidence that BDNF may play a role in the pathogenesis of cardiovascular disease (Chaldakov *et al*., 2004; Cai *et al*., 2006), BDNF serum concentrations were not associated with prevalent cardiovascular diseases or the metabolic syndrome. A closer look on previous studies of BDNF in cardiovascular disease suggests that positive results can be explained by methodological differences. First, some studies have reported an elevated expression of BDNF in atherosclerotic plaques (Ejiri *et al*., 2005), but to date it is not clear to what extent BDNF-expression in atherosclerotic plaques contributes to overall BDNF serum concentrations. Secondly, others have reported lower plasma BDNF concentrations in patients in the acute phase of coronary syndromes (Manni *et al*., 2005), which may be explained by both the acute phase of the coronary syndrome as well as the differences between BDNF measurements in plasma versus serum. The lack of an association between BDNF serum concentrations and prevalent cardiovascular disease is relevant for future studies on the role of BDNF serum concentrations in late-life depression and neurodegenerative disorders in which cardiovascular diseases are highly prevalent. Lung diseases were not associated with BDNF level either. Elevated serum BDNF concentrations have been reported in patients with asthma (Lommatzsch *et al*., 2005), but even when we investigated asthma and Chronic Obstructive Pulmonary Disease (COPD) separately, no effect was found (data not shown).

Strengths and weaknesses

Although we conducted the largest study to date on serum BDNF concentrations in human and included a large number of possible determinants, one important methodological issue has to be addressed for proper interpretation. This issue is that results for serum BDNF concentrations cannot be generalized to studies of BDNF in plasma or platelets. Because of the storage of BDNF in platelets, the concentration of BDNF in serum and plasma differs by a factor of 200 (Rosenfeld *et al*., 1995). As BDNF in platelets does not originate from mega-karyocytes or other precursor cells of the mature

platelet (Fujimura *et al*., 2002), it is likely that most of the BDNF in human platelets is sequestered from blood (Nakahashi *et al*., 2000). Furthermore, BDNF is released by platelets during the clotting process (Rosenfeld *et al*., 1995). This means that differences in platelet functioning, either by their ability to release BDNF or sequester BDNF from blood, may result in differences between serum and plasma BDNF concentrations. A possible disadvantage of measuring BDNF in serum may be a decline in BDNF concentrations after long-term storage of serum, which may not occur for BDNF stored in platelets (Trajkovska *et al*., 2007). In our study, serum was stored at -85 C˚ for a period varying between 22 and 60 months. Although we have found that serum BDNF concentrations significantly declines in long-term storage, this decline was relatively small and was corrected for. Furthermore, only one sample had a BDNF level below the lower detection limit. Therefore, we can conclude that reliable conclusions can be made after a long-term storage at -85 C˚.

Final conclusions

Future studies on serum BDNF concentrations in humans should correct for the time of day of blood withdrawal, storage, age, gender, urbanicity, smoking status and alcohol use. Although effect sizes are generally small and clinical relevance needs to be tested in subsequent clinical samples, we would suggest to exclude subjects who did not adhere to the pretest fasting protocol from clinical studies and to keep storage time limited. Moreover, for diseases with a diurnal variation, like mood disorders, it would be interesting to examine whether other parameters of BDNF serum concentrations (*e.g.,* change of BDNF over the day) would be of more relevance than one measure in the morning.

CHAPTER 3

BDNF concentrations show strong seasonal variation and are correlated with the amount of ambient sunlight

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SIGNIFICANCE: This study shows strong evidence for seasonality in serum BDNF concentrations. This unique finding provides avenues to understand those factors that regulate BDNF expression and they are of tremendous importance in the design- and evaluation of studies on BDNF.

ABSTRACT

Earlier findings show seasonality in processes and behaviors such as brain plasticity and depression that in part are regulated by Brain-Derived Neurotrophic Factor (BDNF). Based on this we investigated seasonal variation in serum BDNF concentrations in 2,851 persons. Analyses by month of sampling (monthly *n*'s > 196) showed pronounced seasonal variation in serum BDNF concentrations ($P < .0001$) with increasing concentrations in the spring-summer period (standardized regression weight (ß) = 0.19, $P < .0001$) and decreasing concentrations in the autumn-winter period (ß = -0.17, $P < .0001$). Effect sizes (Cohen's *d*) ranged from 0.27 to 0.66 for monthly significant differences. We found similar seasonal variation for both sexes and for persons with a DSM-IV depression diagnosis and healthy control subjects. In explorative analyses we found that the number of sunshine hours (a major trigger to entrain seasonality) in the week of blood withdrawal and the 10 weeks prior to this event positively correlated with serum BDNF concentrations (Pearson's correlation coefficients ranged: 0.05 — 0.18) and this could partly explain the observed monthly variation. These results provide strong evidence that serum BDNF concentrations systematically vary over the year. This finding is important for our understanding of those factors that regulate BDNF expression and may provide novel avenues to understand seasonal dependent changes in behavior and illness such a depression. Finally, the findings reported here should be taken into account when designing and interpreting studies on BDNF.

INTRODUCTION

The yearly orbit of the Earth around the Sun causes variability in the length of day. Many species are sensitive to this and exhibit biochemical and behavioral alternations as response. This has been termed seasonality (Walton *et al*., 2011).

Seasonality has become engrained in the field of psychiatry and clinical psychology through findings such as spring peaks in suicide rates (Postolache *et al*., 2010) and seasonal affective disorder (Rosenthal *et al*., 1984, Lewy *et al*., 2006). Above this, subtle effects of season on depressive behaviors have been described. For instance, in periods in which there is relatively little daylight otherwise healthy individuals show reduced levels of activity, less interest in sex, and an increased urge for sleep (Kasper *et al*., 1989; Wehr and Rosenthal, 1989). Research at the pre-clinical level confirmed these findings by showing similar seasonal patterns in depressive-like behavior in rodents (Pyter and Nelson, 2006;; Prendergast and Kay, 2008) and, in addition, in brain plasticity (Pyter *et al*., 2005; Workam *et al*., 2009) that has been linked to the human depressed state (Castren *et al*., 2007). Taken together, these findings suggest that depressive behavior and related processes are sensitive to natural occurring environmental cues such as the length of the day.

Although the underlying mechanisms of seasonality in depressive behaviors could not be elucidated yet, some data point to variation in serotonin (5-hydroxytryptamine; 5-HT) expression as being the molecular mechanism that thrives this association (Lambert *et al*., 2002; Praschak-Rieder *et al*., 2008). Indeed, human studies show that central and peripheral 5-HT activity undergoes marked seasonal rhythmicity (Carlsson *et al*., 1980; Sarrias *et al*., 1989; Hanna *et al*., 1998; Neumeister *et al*., 2000; Praschak-Rieder *et al*., 2008; Luykx *et al*., 2012). Furthermore, 5-HT activity is positively related to the number of ambient sunshine hours (Lambert *et al*., 2002). However, although linked to depression, it has become increasingly clear that 5-HT alternations are not sufficient to cause the primary depressive phenotype (Nestler *et al*., 2002; Sharp and Cowen, 2011). So, seasonality in depressive behaviors might depend on changes in a pathway down-stream of 5-HT rather than directly through 5-HT signaling. Brain-Derived Neurotrophic Factor (BDNF) could serve as a component in such a pathway. BDNF is a signaling molecule that has a repertoire of regulatory functions on a related set of phenomena that are (partly) seasonal (*e.g.,* energy balance and brain plasticity (Tapia-Arancibia *et al*, 2008; Liao *et al*., 2012)). It is well established that 5-HT and BDNF interact with each other and it has been suggested that these factors together have regulatory functions in neuronal functioning and neuronal plasticity (Mattson *et al*., 2004). Furthermore, the intertwined relationship between 5-HT and BDNF plays a fundamental role in the '*neurotrophin hypothesis of depression*' (Duman and Monteggia, 2006). This hypothesis has become a leading model in the field of depression research by conceptualizing depressive disorders as being partly the consequence of deficiencies in mechanisms related to neuronal plasticity. Furthermore, the neurotrophin hypothesis predicts that BDNF and its receptor Tyrosine kinase factor B (TrkB) are targets of antidepressants because these factors modulate neuroadaptive changes that are believed to be essential for therapeutic change (Castren *et al*., 2007).

Given seasonality in 5-HT dynamics, the intrinsic relation between 5-HT and BDNF, and links between BDNF and processes and behaviors that occur according to a seasonal pattern, serum BDNF concentrations may also follow a seasonal pattern. A line of experimental work that has shown that light deprivation reduces BDNF mRNA and protein expression in the rat brain (Castren *et al*., 1992; Karpova *et al*., 2010) also gives ground to this idea. To date, however, no studies investigated seasonality in any BDNF parameter. Clarifying this issue is important in understanding the factors that influence BDNF expression, essential in evaluating research findings, and maybe helpful for a better understanding of seasonal variation in depressive-like behavior. Accordingly, we investigated seasonality in serum BDNF concentrations that are assumed to reflect central levels of BDNF (Pan *et al*., 1998; Klein *et al*., 2010). Since seasonality is entrained by environmental cues such as the number of sunshine hours, we also explored the relation between serum

BDNF concentrations and this variable. Here we hypothesized that BDNF concentrations will be positively related to the number of sunlight hours, as has been shown for 5-HT expression (Lambert *et al*., 2002).

METHODS

Subjects

Subjects were derived from the baseline sample of the Netherlands Study of Depression and Anxiety (NESDA). The NESDA is an ongoing cohort study among 2,981 persons who were recruited in mental health care, primary care and in the general population. Included in the NESDA were persons with a depressive and/or an anxiety disorder, persons with a depressive and/or an anxiety disorder in remission and persons without lifetime depressive or anxiety disorders. Exclusion criteria were evidence for psychotic-, bipolar I- or II-, or obsessive-compulsive disorder, severe alcohol use, and not being fluent in Dutch (see Penninx *et al*., 2008 for full details). For our present purposes, subjects were eligible on whom data on serum BDNF concentrations and date of blood withdrawal were available (N = 2,851, not eligible n = 130 [~ 4.5%]). Eligible and not-eligible persons did not differ with regard to gender, age and psychiatric diagnoses (all P values > .25). Ethical approval for the study was obtained from the Ethical Committees of the participating Institutes and all subjects gave their written informed consent.

Demographical, behavioral, and clinical measurements

Basic demographic and behavioral information on the sample was acquired through standard questions and procedures (see Penninx *et al*., 2008). Information on the amount of physical activity the participants engaged in was acquired by means of the International physical activity questionnaire and expressed as the number of met-minutes (*i.e.,* the ratio of the amount of energy expenditure during activity to the energy expenditure at rest; Craig *et al*., 2003). Smoking was dichotomized in current smoker versus non-current smoker and alcohol use as abstainer versus non-abstainer.

Trained staff administered the Composite International Diagnostic Interview version 2.1 (CIDI; Wittchen *et al*., 1991) to establish lifetime and current (*i.e.,* a diagnoses in the past 6-months) psychiatric diagnoses (Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV; American Psychiatric Association, 1994). The severity of depressive and anxiety symptoms was assessed using the Inventory of Depressive Symptoms (IDS; Rush *et al*., 1996) and Beck's Anxiety Inventory (BAI; Beck *et al*., 1979). The use of antidepressants was assessed by means of self-report and drug container observation. Antidepressant use was defined as intake of minimally the daily dose as recommended by the World Health Organization (2008) on > 50% of the days during the last month.

BDNF measurements

Fifty ml of venous blood was withdrawn into vacuum tubes between 07:30 and 09:30 a.m. after an overnight fast (from August 2004 to March 2007). Serum was separated immediately and stored until assay. Serum BDNF concentrations were determined, in a laboratory of the University of Maastricht, the Netherlands, using the Emax Immuno Assay system from Promega according to the manufacturer's protocol (Madison, WI, USA). Absorbency was read in duplicate using a Bio-Rad Benchmark microplate reader (Hercules, CA, USA) at 450 nm. Serum BDNF concentrations were expressed as ng/ml. The coefficients of variance ranged between 2.9% and 8.1%. Further information on the exact procedures (and their reliability and validity) that were used is provided elsewhere (Bus *et al*., 2011).

Sunlight measurements

The number of sunlight hours was measured using pyranometers (World Meteorological Association (WMO; 1996), on a daily basis, at weather stations of the Dutch Royal Meteorological Institute (KNMI 2012; www.knmi.nl). The weekly number of sunlight hours was defined as the sum of all sub-periods in that week for which the solar irradiance exceeded 120 W/m^2. Since NESDA gathered data at multiple sites in the Netherlands (*i.e.,* Amsterdam, Groningen, and Leiden) data on the number of sunlight hours were collected from the weather stations that were most nearby the study sites (*i.e.,* Schiphol weather station [latitude: 52˚18'N] for the Amsterdam and Leiden areas [latitudes: 52˚31'N and 52˚09'N respectively] and Eelde weather station [latitude: 53˚08'N] for the Groningen area [latitude: 53˚12'N]).

Statistical analyses

Data were analyzed in SPSS 18.0 (Chicago, IL, USA) and are presented as the average ± standard deviation, unless otherwise indicated. Seasonal and monthly differences in demographical, behavioral, and clinical variables were analyzed using a χ^2 (categorical variables) or Fisher's exact test (continuous variables). Statistical significance was set at $P < .05$ (two-tailed). Effect sizes were reported as standardized Cohen's *d*, standardized regression weights (ß), or eta-squared (η^2) where appropriate.

Analysis of covariance was used to test for differences in serum BDNF concentrations by calendar month of sampling. The following covariates were included: gender, ethnicity, age, BMI, time of the day of blood draw, duration of serum storage, a current depression and/or anxiety diagnosis, antidepressant use, and sampling site because these variables have been shown to affect serum BDNF concentrations (Bus *et al*., 2011; Molendijk *et al*., 2011). This was followed up by Bonferroni corrected *post-hoc* tests when required. In view of earlier findings (Bus *et al*., 2011; Molendijk *et al*., 2011), three additional sub-group analyses were performed with gender, psychiatric status, and antidepressant treatment status as additional grouping factors in order to assess potential interaction effects between these variables and month of sampling. In a similar manner we tested whether the earlier reported lower BDNF concentrations in antidepressant free depressed persons as compared to healthy control subjects, remitted depressed persons, and antidepressant treated depressed persons (Molendijk *et al*., 2011) remained statistically significant when month of sampling was controlled for.

Associations between the number of sunshine hours in the week of blood withdrawal and serum BDNF concentrations were explored using zero-order and partial Pearson's product-moment correlation coefficients (*r*). For the latter, the variance due to the set of covariates (see above) was taken into account. We anticipated that sunlight related BDNF alternations could occur with some time delay and therefore we not only related BDNF concentrations to the number of sunshine hours in the week of blood withdrawal, but also to the number of sunshine hours in the weeks prior to blood withdrawal. Finally, the variables coding for the number of sunshine hours in the week of blood withdrawal and the 10 weeks prior to this event, were entered together in 1 block of a multiple stepwise regression analysis (the set of covariates was entered in a first block) to test the cumulative effect of the number of sunshine hours in the recent past on serum BDNF concentrations.

RESULTS

The overall sample (*N* = 2,851) had a mean age of 41.8 ± 13.1 years and included 1,827 women (66.5%). **Table 1 ↓** shows the characteristics of the study participants by season of sampling (for a table on the characteristics of the study participants by month of sampling please contact Marc Molendijk; molendijkml@fsw.leidenuniv.nl). Subtle seasonal differences were observed in measures for psychiatric diagnoses and depression severity. These differences were similar to the findings reported by Winthorst *et al.* (2011) who studied seasonal variation of depressive and anxiety symptoms using, in part, the same data.

Shortly, there was a small rise in the winter season in the percentage of clinical diagnoses of depression and depression-anxiety and related to this, on average higher scores on the IDS and a slightly higher number of persons who used an antidepressant. Next to this, persons who were sampled in the winter tended to have a somewhat higher BMI (all differences < 1 BMI point) as compared to persons who were sampled in the other seasons.

Table 1. Descriptive information on the sample (mean ± *STD* or percentages [*n*]) by season of sampling

	Spring Mar 21–Jun 20 (*n* = 693)	**Summer** Jun 21–Sept 20 (*n* = 663)	**Autumn** Sept 21–Dec 20 (*n* = 815)	**Winter** Dec 21–Mar 20 (*n* = 680)	*P*-value
Females	67.3% [466]	65.0% [431]	65.2% [531]	67.9% [462]	= .54
Age	41.5 ± 13.2	41.0 ± 13.3	42.5 ± 13.1	42.3 ± 12.5	= .08
Northern European ancestry	95.7% [663]	94.3% [625]	94.1% [766]	95.1% [647]	= .49
Education (years)	12.3 ± 3.3	12.2 ± 3.3	12.1 ± 3.3	12.0 ± 3.2	= .40
Body Mass Index	25.3 ± 4.5	25.7 ± 5.1	25.3 ± 5.1	26.1 ± 5.3	< .05 [a]
Smoker	37.3% [258]	34.6% [229]	34.2% [279]	33.2% [226]	= .43
Physical activity [1]	3.6 ± 3.1	3.7 ± 3.2	3.4 ± 3.1	3.4 ± 3.2	= .33
Wake up time	06:56 ± 49.3	06:58 ± 40.5	06:59 ± 44.1	06:54 ± 48.1	= .11
Time of blood draw	08:48 ± 17.4	08:47 ± 20.4	08:49 ± 33.3	08:59 ± 22.8	= .77
Psychiatric status					
Healthy controls [2]	43.9% [304]	45.2% [300]	47.1% [384]	36.7% [250]	< .001 [b]
MDD [2]	12.3% [85]	12.2% [81]	11.2% [91]	16.0% [108]	< .05 [a]
Anxiety [2, 3]	20.5% [142]	18.3% [121]	17.5% [143]	21.0% [143]	= .23
Comorbid anxiety [2, 3]	23.4% [162]	24.3% [161]	24.2% [197]	26.3% [179]	= .63
Antidepressant medication [4]	23.4% [162]	22.2% [147]	24.9% [203]	28.2% [192]	= .05
Depression severity, IDS	21.2 ± 14.5	20.1 ± 14.5	20.9 ± 15.0	22.8 ± 13.9	< .01 [a]
Anxiety severity, BAI	12.1 ± 10.7	11.6 ± 10.5	12.0 ± 11.5	12.6 ± 10.5	= .52
Alcohol Use [5]					
Non-drinker	46.4% [322]	50.9% [337]	52.2% [425]	49.5% [337]	= .21
Drinker 0-1 unit a day	37.2% [258]	36.7% [243]	36.4% [297]	37.6% [256]	= .97
Drinker 1-2 units a day	10.6% [73]	7.7% [51]	7.1% [58]	6.9% [47]	= .08
Drinker > 2 units a day	5.9% [41]	4.8% [32]	4.2% [34]	5.9% [40]	= .44

Abbreviations: BAI, Beck's Anxiety Inventory; IDS, Inventory of Depressive Symptoms; MDD, Major Depressive Disorder.

[1] Mean met-minutes (*i.e.,* ratio of energy expenditure during activity to energy expenditure at rest).

[2] Current (6 months diagnosis).

[3] Included a diagnosis of social phobia, panic disorder, generalized anxiety disorder, and/or agoraphobia.

[4] Included the use of SSRI, SNRI, TCA, NaSSA, and/or St. John's worth for at least one month at regular dose.

[5] Beyond normal alcohol use, binge drinking (> 6 units at 1 occasion) was most often reported in the spring

[a] Post-hoc tests showed higher levels in the winter as compared to the other seasons

[b] Post-hoc tests showed higher levels in the summer as compared to the other seasons

Seasonality in serum BDNF concentrations

We found strong indications for a seasonal pattern in serum BDNF concentrations (overall $F = 11.32$, $P < .0001$, $\eta^2 = 0.04$, see **Figure 1 ↓**). The seasonal pattern was such that serum BDNF concentrations were, on average, lower in the months January to May and higher in the months June to December. For pair-wise comparisons by month of sampling see **Table S2** in **Appendix II**. Effect sizes for monthly differences that were statistically significant after Bonferroni corrections ranged from small ($d = 0.27$, January versus August) to large ($d = 0.66$, March versus September).

A similar seasonal pattern in serum BDNF concentrations was observed for males and females, depressed and non-depressed persons, and for antidepressant treated (any antidepressant) and

antidepressant free persons (data not shown). The *P*-values for the interaction terms between the additional grouping factors and month of sampling were all > .10. Given our earlier findings showing that serum BDNF concentrations were highest in currently depressed persons on selective serotonin reuptake inhibitors or St John's wort (Molendijk *et al*., 2011) we in addition tested for seasonality in serum BDNF concentrations in persons using these agents. A similar seasonal effect in serum BDNF concentrations was observed ($\eta^2 = 0.074$) in this particular group.

Nadir (March) and peak (September) BDNF concentrations showed a correspondence with the end of the winter and the summer respectively. Even more, the pattern of BDNF concentrations over the year suggested an increase during the Dutch spring-summer period (equinox vernal, March 20 to September 22) and a decrease in the Dutch autumn-winter period (equinox autumnal, September 23 to March 19). Indeed, when decomposed by equinox, we observed a coherent pattern of increasing BDNF concentrations during the spring and summer (*n* = 1,356, ß = 0.19, *P* < .0001) and decreasing BDNF concentrations during the autumn and winter (*n* = 1,495, ß = -0.17, *P* < .0001, *t* = 9.87 [*P* < .0001] for the interaction equinox of sampling - consecutive week of sampling within the equinox; see supplementary **Figure S1** in **Appendix II**).

Although somewhat attenuated, the earlier reported main effects (Molendijk *et al*., 2011) of lower serum BDNF concentrations in antidepressant free depressed persons as compared to healthy control subjects, remitted depressed persons, and antidepressant treated depressed persons remained statistically significant (*P* = .04, *P* < .001, and *P* = .04) in analyses in which diagnostic and treatment status were added as grouping factors next to month of sampling.

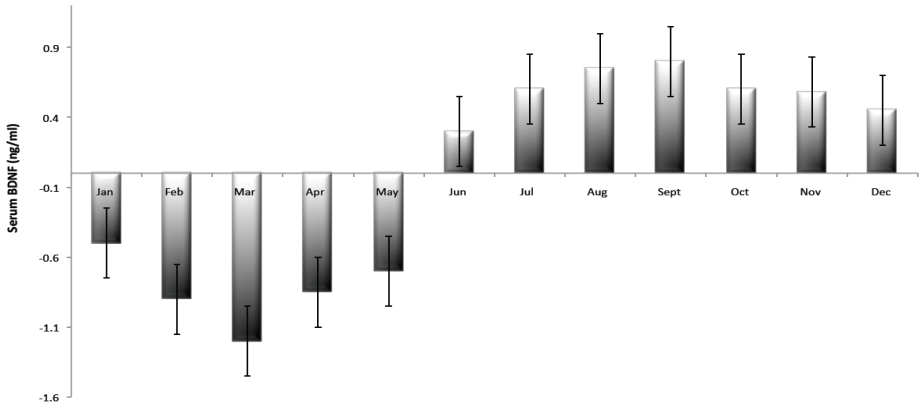

Figure 1. Mean serum BDNF concentrations by month of sampling.

Error bars reflect the *SEM*.

For pair-wise comparisons on serum BDNF levels by month of sampling I refer to **Appendix II, Table S1**.

Explorative analyses: sunshine hours and serum BDNF concentrations

The number of sunshine hours per week throughout the year ranged from 2 hours per week to 131 hours per week. Exploratory analyses showed small positive correlations between the number of sunshine hours (in the week of blood withdrawal, and the 10 weeks prior to that) and serum BDNF concentrations ([zero order and partial] in the range: *r* = 0.04 — *r* = 0.18; see supplemental material **Table S2** and **Figure S2** in **Appendix II**). Correlations were largest for the number of sunlight hours 7 and 8 weeks prior to blood withdrawal. Together, the number of weekly sunlight hours in the week of sampling and in the 10 weeks prior to the week of sampling, positively predicted serum BDNF concentrations (entered in 1 block of a multiple stepwise regression analysis (the set of covariates was entered as a first block): Δ*F* = 11.22, multiple correlation = 0.21, *P* < .0001). The number of sunshine hours could partly explain the monthly differences in serum BDNF concentrations (i.e., $\eta^2 = 0.041$, *P* < .0001 before and $\eta^2 = 0.011$, *P* = .003 after the inclusion of the number of sunlight hours as covariates). Additional explorative analyses showed a similar pattern of correlations between the length of day (*i.e.,* photoperiod) in the week of blood withdrawal, and the 10 weeks prior to that, and serum BDNF concentrations (data not shown).

DISCUSSION

The novel finding that emerges from this study is that of pronounced seasonal variation in serum BDNF concentrations. The seasonality that we observed followed a coherent pattern of increasing BDNF concentrations over the course of the spring and the summer and decreasing BDNF concentrations over the course of the autumn and the winter. Illustrative for the robustness of our finding was that the seasonal pattern was similar for both sexes and for conditions in which BDNF expression is altered, such as major depression (Duman *et al*., 2008). By means of covariate adjusted analyses we could exclude a range of potentially alternative explanations for our findings, including effects of for example physical activity and BMI that have been reported to be seasonal (Ma *et al*., 2006; Gordon *et al*, 1987) and that have been linked to serum BDNF concentrations as well (Currie *et al*., 2009; Golden *et al*., 2010). In addition, our results were of a relatively large magnitude with effect sizes (Cohen's *d*) up to 0.66 for monthly differences. Together, these findings may have significant implications as discussed below.

Seasonality in serum BDNF concentrations: mechanisms and theoretical implications

What might explain the strong pattern of seasonality in serum BDNF concentrations? Differences in cAMP-response element binding protein (CREB) activity could be an option. CREB is a transcription factor that binds to the promoter region of the BDNF gene and positively regulates BDNF transcription (Impey *et al*., 2004). CREB activity is under the influence of 5-HT. Given that long day conditions give rise to a higher 5-HT expression (Lambert *et al*., 2002), seasonality in serum BDNF concentrations may be entrained by 5-HT induced CREB activation. This explanation would fit with observations that come from the field of antidepressant research. For example, increases in the transcriptional activity of BDNF in the course of treatment with a selective serotonin reuptake inhibitor are often observed but these only occur in the face of an increase in CREB activity (Conti *et al*., 2002). Also, antidepressants give rise to increased CREB activity but only if they are administered chronically (*e.g.,* ≥ 21 days) (Conti *et al*., 2002). This appears to be in agreement with our observation that the correlations between BDNF concentrations and the number of sunshine hours were largest for the number of sunshine hours in 7 to 8 weeks prior to blood withdrawal. Furthermore, peak serum BDNF values were observed in the early autumn and nadir values in the early spring. So, the seasonality in BDNF expression seems to occur with a time-delay relative to the seasons and their corresponding weather characteristics.

Important for the interpretation of our results is the question whether peripheral differences in serum BDNF concentrations imply that there also are differences at the level of the Central Nervous System (CNS). As already shortly mentioned in the introduction part of our paper, it has been shown in rodents that BDNF crosses the blood-brain barrier (Pan *et al*., 1998) and that peripheral and central BDNF concentrations correlate positively (Klein *et al*., 2010). Therefore it is reasonable to assume that blood BDNF concentrations reflect BDNF concentrations in the CNS. Unfortunately the literature on this topic in humans is limited to only one study in which blood simultaneously was derived from high up the jugular veins and from arterial veins (Dawood *et al*., 2007). The results of this study showed that BDNF levels were higher in blood that was derived from the internal jugular veins as compared to arterial blood. This indeed is indicative for CNS production of BDNF that is obtained from peripheral tissues (Dawood *et al*., 2007). It should be noted though that some studies report null-findings with regard to an association between peripheral BDNF concentrations and more central parameters for BDNF activity, that is, the absence of correlations between plasma and cerebral spinal fluid concentrations of BDNF (Pillai *et al*., 2012).

Seasonality in serum BDNF concentrations: practical implications

Notwithstanding some uncertainty with regard to the exact mechanisms underlying the seasonality in BDNF expression, our findings have immediate and important practical implications. First, our findings are of essential importance in the interpretation of results from longitudinal studies. That is, the results of trials that have serum BDNF concentrations as an outcome measure and that span several months, might be of little use unless detailed knowledge on seasonal effects is available. Second, and in agreement with the latter statement, our results stress the need to sample groups (*e.g.,* depressed patients versus healthy control subjects) equally over the year in order to gain credibility and validity in research findings.

Strengths, Limitations, and future studies

Our study has strengths, notably reliability through a large sample size and validity through the adjustment for a range of confounders and subgroup analyses. However, there are also limitations. First, we used between-subject data whereas within-subject data with repeated samplings (monthly or more often) over at least 1 calendar year is more appropriate to establish seasonality. Second, there must have been some noise in the measurements related to the variables that coded for the hours of sun in the weeks prior to blood withdrawal. That is, we assigned each individual the number of sun hours that were recorded in the particular region these persons lived in under the assumption that they actually were in that particular region. However, we do not know this with certainty (*e.g.,* participants may have been on vacation) and we also do not know whether they truly were exposed to the sun (*e.g.,* participants may have stayed inside their houses). We assume that this resulted in random noise that decreased our ability to detect associations and thus that the true associations between sunlight hours and serum BDNF concentrations are likely to be of a larger magnitude than as reported here. Also, from our data we cannot conclude whether serum BDNF concentrations are kept in a certain homeostatic range over the year to serve a given function or whether we observed an epiphenomenon of some other physiological or (unmeasured) behavioral process that may be unrelated to central BDNF functioning. Finally, a limitation with regard to the Promega BDNF ELISA kit that was used is that it quantifies total amount of BDNF without distinguishing between the pro- and the mature BDNF forms (Lu *et al*., 2005; Yoshida *et al*., 2012 a). It could well be that the percentage of mature BDNF to total BDNF in serum may be altered as a function of season. Only since very recently it has been shown that such an important distinction can be made (Yoshida *et al*., 2012 a, b) and thus future studies could look into differences in pro-mature BDNF ratios over the seasons.

For future studies it further would be interesting to elucidate whether tryptophan, 5-HT, and/or CREB activity, truly have important roles, as hypothesized here, in the chain of events that in the end may lead to seasonality in serum BDNF concentrations. It also would be particularly interesting to study BDNF concentrations as a function of selected levels of (sun)light exposure. From our findings we would expect that serum BDNF concentrations will vary less in areas where the seasons, the number of sunlight hours, and/or the natural length of day vary less over the year (e.g., closer to the equator as compared to the Netherlands) or 12 hours light/dark regimens in laboratories. Finally, in the light of our findings that serum BDNF concentrations are correlated with the amount of ambient sunlight (and with the length of the photoperiod) an important area of investigation would be to investigate changes in serum BDNF concentrations in the course of treatment with bright light (Lieverse *et al*., 2011) in conditions that have been associated with an altered BDNF expression.

Summary and conclusions

Here we demonstrate that serum BDNF concentrations profoundly vary over the year and that this occurs according to a coherent pattern of increasing BDNF concentrations in the spring-summer period and decreasing BDNF concentrations in the autumn-winter period. In addition we show correlations between

serum BDNF concentrations and the number of sunlight hours. Although much remains to be understood with regard to these associations and notwithstanding some limitations, we believe that these results invite for a perspective on BDNF related mechanisms in which seasonality is engrained.

CHAPTER 4

Serum BDNF concentrations in major depressive disorder: state-trait issues, clinical features and pharmacological treatment

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SIGNIFICANCE: This paper reveals that low serum BDNF concentrations are a state characteristic of depression and that this normalizes following natural remission and in the course of antidepressant treatment. Critically however is that we show that the effect-sizes on these associations are small and that normalization of serum BDNF concentrations is not necessarily associated with a relief of depressive symptoms.

ABSTRACT

Recent evidence supports *the neurotrophin hypothesis of depression* in its prediction that Brain-Derived Neurotrophic Factor (BDNF) is involved in depression. However, some key questions remain unanswered, including whether abnormalities in BDNF persist beyond the clinical state of depression, whether BDNF concentrations are related to the clinical features of depression and whether distinct antidepressants affect BDNF concentrations equally. We addressed these questions and investigated serum BDNF concentrations in 962 depressed patients, 700 fully remitted persons (> 6 months) and 382 healthy controls. We found serum BDNF concentrations to be low in antidepressant-free depressed patients relative to controls (effect size, Cohen's *d*) = 0.19, $P < 0.01$) and to depressed patients who were treated with an antidepressant ($d = 0.23$, $P < 0.01$). BDNF concentrations of fully remitted persons (whether unmedicated or treated with an antidepressant) were comparable to those of healthy controls. Analyzing the sample of antidepressant-free depressed patients showed that BDNF concentrations were unrelated to the core clinical features of depression such as its severity or having a first versus a recurrent episode. The to antidepressant use associated up-regulation of serum BDNF concentrations in depressed patients was confined to selective serotonin reuptake inhibitors ($d = 0.39$, $P < 0.01$) and St John's wort ($d = 0.63$, $P = 0.03$). Our results suggest that low serum BDNF concentrations are a state abnormality that is evident during depression and that normalizes during remission. Increases in serum BDNF concentrations during antidepressant treatment appear to be confined to some antidepressants and do not parallel clinical characteristics, such as the severity of depressive symptoms.

INTRODUCTION

Brain-Derived Neurotrophic Factor (BDNF) is a neurotrophin that has been linked to the viability of neurons in brain circuits that regulate emotion, memory, learning, sleep and appetite (Duman *et al*., 2000; Sutton and Schuman, 2006). The neurotrophin hypothesis of depression is based on these functions of BDNF and postulates that depression results from stress-induced decreases in BDNF expression and that antidepressants are efficacious because they increase BDNF expression (Duman *et al*., 1997; Duman and Monteggia, 2006). Consistent with this hypothesis are the findings that depression is accompanied by decreased central and peripheral concentrations of BDNF (Sen *et al*., 2008), and that antidepressants elicit an increase in BDNF concentrations in animal models for depression (Angelucci *et al*., 2005) and in depressed humans (Brunoni *et al*., 2008). Together with the latency of weeks before antidepressants become clinically effective (Nemeroff and Owens, 2002), these observations shaped the hypothesis that the efficacy of antidepressants depend on neuroadaptive changes that are brought about by changes in BDNF signaling (Duman and Monteggia, 2006).

Taken together, there is reason to believe that BDNF is involved in depression and in antidepressant action. Results inconsistent with the neurotrophin hypothesis, however, also have been reported. There are, for example, studies that did not detect alternations in BDNF in depressed persons or in the course of treatment with an antidepressant (Matrisciano *et al*., 2009). In addition, some questions remain unanswered so that the neurotrophin hypothesis is at best incomplete (Groves, 2007). A major question that needs to be answered is whether low BDNF concentrations persist beyond the clinical state of depression (Trajkovska *et al*., 2008). A second question is whether BDNF concentrations are related to the clinical features of depression, such as having a first versus a recurrent episode (Lee *et al*., 2007). Yet a third outstanding question is whether all classes of antidepressants affect BDNF concentrations equally. We therefore studied, cross-sectionally, serum BDNF concentrations of depressed patients, remitted depressed persons and never depressed persons. Our efforts had three concerns: (1) to compare serum BDNF concentrations of antidepressant-free and antidepressant treated current and fully remitted depressed patients and never depressed persons, (2) to explore the associations between some of the core clinical features of depression and serum BDNF concentrations and (3) to evaluate the association between the use of several distinct classes of antidepressants and serum BDNF concentrations.

METHOD

Patients and sample collection

Patients were from the Netherland Study of Depression and Anxiety (NESDA). Full details on the rationale, objectives and protocol of NESDA are described in Penninx *et al*. (2008). In brief, NESDA is a prospective cohort study (*N* = 2,981) that recruited patients in mental health care, primary care and in the general population. Included were persons with a depressive and/or an anxiety disorder, persons with a depressive and/or an anxiety disorder in remission and persons without a history of these disorders. Persons who were diagnosed with a psychotic disorder, bipolar disorder (type I and II), obsessive-compulsive disorder, or severe alcohol use disorder were not eligible. Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) diagnoses (APA, 1994) were assigned on the basis of responses to the Composite International Diagnostic Interview 2.1 lifetime version (CIDI 2.1; Wittchen *et al*., 1991) that was administered by trained interviewers. At baseline, participants provided blood samples, underwent a medical examination and gave written informed consent for the study that was approved by the Ethical Committees of the participating institutes.

Our study enrolled 2,044 persons (68.6% of the NESDA sample). On the basis of the assigned diagnosis, antidepressant use and the availability of BDNF data, we created five groups: antidepressant-free

depressed patients (*n* = 541), antidepressant-treated depressed patients (*n* = 421), antidepressant-free remitted depressed persons (*n* = 539), antidepressant-treated remitted depressed persons (*n* = 161) and healthy persons who served as controls (*n* = 382). Depressed patients met the criteria for a depressive episode the last 6 months (*n* = 541). The majority of these patients had a current diagnosis of depression (*n* = 388), but some (*n* = 153) had a diagnosis of depression 1–6 months prior to baseline and did not fulfill all criteria in the past month. Persons who were in full remission of depression were diagnosed with major depression somewhere in their lives, but had been free of depression and anxiety for at least 6 months. Persons were included in the control group when they had: (1) no lifetime mood or anxiety disorders, (2) no documented family history of depression or anxiety and (3) a low score (< 14) on the Inventory of Depressive Symptoms (IDS; Rush *et al*., 1996).

Data on antidepressants use were acquired through drug container observation and self-report. Use of an antidepressant was defined as intake of minimally the daily dose as recommended by the WHO (2010) during the last month on at least 50% of the days. We coded for the use of selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), noradrenergic and specific serotonergic antidepressants (NaSSAs) and St John's wort (Hypericum perforatum). The duration of antidepressant use was expressed in months.

Clinical features of depression

All patients were characterized on the symptom severity of depression using the IDS (Rush *et al*., 1996). Patient samples were further characterized on having a first or a recurrent depressive episode, the presence of comorbid anxiety, the age at onset of depression, the recency of depression, the chronicity of depression and on the presence of suicide ideation. The CIDI (Wittchen *et al*., 1991) served as source of information on the presence of a first- or a recurrent depressive episode, the presence of a comorbid anxiety, age at onset of depression (*i.e.,* the age at which the first episode occurred) and the recency of depression (*i.e.,* fulfilling criteria in the past month versus fulfilling criteria in the past 6 months but not in the past month). Depression was considered chronic if symptoms had been present for more than 24 months during the last 5 years, which was assessed using the life chart method (Lyketsos *et al*., 1994). The scale for suicide ideation (Beck *et al*., 1997) was used to examine the presence of suicide ideation.

BDNF measurements

A measure of 50 ml of blood was withdrawn into vacuum tubes between 0730 and 0930 hours after an overnight fast. After blood collection, serum was separated and stored at -85 C˚ until it was assayed. BDNF protein concentrations were measured using the Emax Immuno Assay system from Promega according to the manufacturer's protocol (Madison, WI, USA), in one laboratory by one technician who was blinded for the clinical diagnoses of the participants. Undiluted serum was acid treated as this reliably increased the detectable amount of BDNF in a dilution-dependent way. Greiner Bio-One high affinity 96-well plates were used. Serum samples were diluted 100 times, and the absorbency was read in duplicate using a Bio-Rad (Hercules, CA, USA) Benchmark microplate reader at 450 nm. The intra- and inter-assay coefficients of variation were found to be within 3 and 9%, respectively. Four persons had BDNF serum concentrations that were below the reliable detection threshold of 1.56 ng ml. These values were set at the lower detection limit. Positive outliers (mean **>** 3 SD, *n* = 6) were trimmed to the mean + 3 *SD* value. There were no differences between persons with missing and non-missing BDNF with regard to gender (*P* = 0.71), age (*P* = 0.67) and diagnoses (*P* = 0.33).

Covariates

Potential variance due to gender, age and educational level was controlled for in all analyses. In addition, we controlled for body mass index (BMI), physical activity and smoking as these variables are associated with BDNF (Suwa *et al*., 2006; Rojas-Vega *et al*., 2006) and mood (Simon *et al*., 2006; Harris *et al*., 2006; Kendler *et al*., 1993). Data on weight and height were collected, and BMI was calculated (weight/height^2). Information on physical activity was gathered using the International Physical Activity Questionnaire (IPAQ; Craig *et al*., 1995) and expressed as the number of met-minutes (*i.e.,* the ratio of the amount of energy expenditure during activity to the energy expenditure at rest). Smoking status was dichotomized as current versus non-smoker. Time of the morning blood withdrawal and duration of serum storage were controlled for since BDNF concentrations vary according to variation on these variables (Begliuomini *et al*., 2008; Trajkovska *et al*., 2007).

Statistical analyses

All computations were performed in SPSS version 17.0 (SPSS, Chicago, IL, USA). BDNF values were controlled for basic covariates in all analyses. Effect sizes on pair-wise comparisons were presented as Cohen's *d*. (Cohen, 1988). A two-tailed *P*-level of 0.05 was used to determine statistical significance.

Analysis of variance (ANOVA) was used to compare BDNF concentrations of antidepressant-free depressed patients and antidepressant-treated depressed patients, antidepressant-free patients and antidepressant- treated persons who were in remission (**<** 6 months) and controls. Post-hoc tests were performed following a significant *F*-statistic using Tukey's test.

A multivariable regression analysis was used to identify whether the clinical features of depression were associated with BDNF concentrations. Regression was performed in patient groups in which mean BDNF concentrations deviated significantly from the control group. Pearson correlation coefficients between predictors and BDNF concentrations were also calculated. Basic covariates were entered in the first step of regression. In the second step, the clinical features of depression were entered. The regression model was fit using method enter. Tolerance of the predictors and normality of error variances was verified.

To establish whether the use of an antidepressant effected BDNF concentrations equally in current and remitted depression, a 2 (currently depressed versus depression in (full) remission) times 2 (antidepressants; yes versus no) ANOVA was performed. Potential antidepressant-specific associations between the use of SSRIs, TCAs, SNRIs, NaSSAs and St John's wort and BDNF concentrations were evaluated by contrasting BDNF concentrations of persons who used one of these agents against the mean BDNF concentration of the antidepressant- free persons. Analyses were repeated with the severity of depressive symptoms and the duration of antidepressant use as covariates.

RESULTS

Demographics and clinical features

Demographical and clinical features among the five groups are given in Table 1. ANOVA and χ^2 tests showed that, compared with controls, depressed and remitted persons were more likely to be female, to be older, to have received fewer years of education and to smoke. BMI was higher in current and remitted antidepressant-treated depressed persons compared with controls and to antidepressant-free depressed and remitted persons. The amount of physical activity was low in the antidepressant treated currently depressed group relative to the other groups. Post-hoc comparisons on demographical and clinical features between the current and remitted depressed groups are given in **Table 1 ↓**.

BDNF concentrations in persons with current or remitted depression and controls

An ANOVA model showed a main effect of diagnostic status on serum concentrations of BDNF ($F_{1,\ 1578}$ =

4.09, *P* = 0.01). Pair-wise comparisons (see **Figure 1 ↓**) indicated that serum BDNF concentrations were low in antidepressant-free depressed patients compared with controls (*d* = 0.19), antidepressant-free persons who were in full remission (*d* = 0.15), and antidepressant-treated depressed patients (*d* = 0.23). BDNF concentrations of antidepressant-free persons who were in full remission and depressed patients who were treated with an antidepressant were comparable to those of controls.

Table 1. Demographic- and clinical characteristics (mean ± *STD* or percentages) of participants by depression diagnosis (never, current and remitted) and antidepressant use (no versus yes AD)

	Controls (*n* = 382)	Depressed (*n* = 541)	Depressed Antidepressant (*n* = 421)	Remitted (*n* = 539)	Remitted Antidepressants (*n* = 161)	*P*-value
Female (%)	61.0	66.7	67.0	71.1	70.8	< 0.05
Age	45.7 ± 12.3	39.8 ± 12.6	42.6 ± 11.0	43.1 ± 12.9	45.4 ± 10.8	< 0.001 [A,B]
Education (years)	13.4 ± 3.3	11.9 ± 3.2	11.7±3.3	12.6 ± 3.1	12.1 ± 3.3	< 0.001
Body mass index	25.4 ± 4.6	25.5 ± 5.4	26.3±5.6	25.3 ± 4.6	26.6 ± 5.6	< 0.01 [A,B]
Physical activity [C]	3.7 ± 3.0	3.5±3.3	3.2±3.3	3.8 ± 3.1	3.1 ± 2.8	< 0.01 [B]
Smoker	16.5	38.7	46.0	35.5	34.3	< 0.001 [A]
Alcohol dependent	5.4	23.3	20.0	17.0	13.7	< 0.001
Depression severity	5.3 ± 3.5	29.6 ± 12.7	34.5 ± 13.1	16.8 ± 10.3	20.3 ± 10.6	< 0.001 [A,B]
Age of onset of MDD	NA	26.1 ± 12.3	27.4 ± 12.6	27.6 ± 12.2	28.2 ± 11.7	0.35
Chronic depression [D]	NA	27.5	38.3	11.1	18.7	< 0.001 [A,B]
>1 episode	NA	63.6	58.2	54.6	61.5	< 0.05 [A]
Comorbid anxiety [E]	NA	42.2	47.7	NA	NA	< 0.05
Suicide ideation	NA	22.4	29.3	5.2	6.2	< 0.001 [A]
Antidepressant medication						
SSRI	NA	NA	62.7	NA	65.8	0.27
SNRI	NA	NA	16.4	NA	13.0	0.06
TCA	NA	NA	8.1	NA	13.0	0.19
NaSSA	NA	NA	8.6	NA	2.5	< 0.05
St John's wort	NA	NA	4.3	NA	5.6	0.32
Duration of use [F]	NA	NA	7.5 ± 4.9	NA	10.9 ± 3.5	< 0.001

Abbreviations: NaSSA, noradrenergic and specific serotonergic antidepressant; SNRI, serotonin and norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant

[A] Indicates a significant difference between the antidepressant treated and antidepressant free current depressed groups

[B] Indicates a significant difference between the antidepressant treated and antidepressant free remitted depressed groups

[C] Mean met-minutes (that is ratio of energy expenditure during activity to energy expenditure at rest) divided by 1000

[D] Symptoms were considered chronic if they were present for at least 24 months during the last 5 years

[E] Included social phobia, panic disorder with and without agoraphobia, agoraphobia and generalized anxiety disorder

[F] Duration of use is expressed in number of months

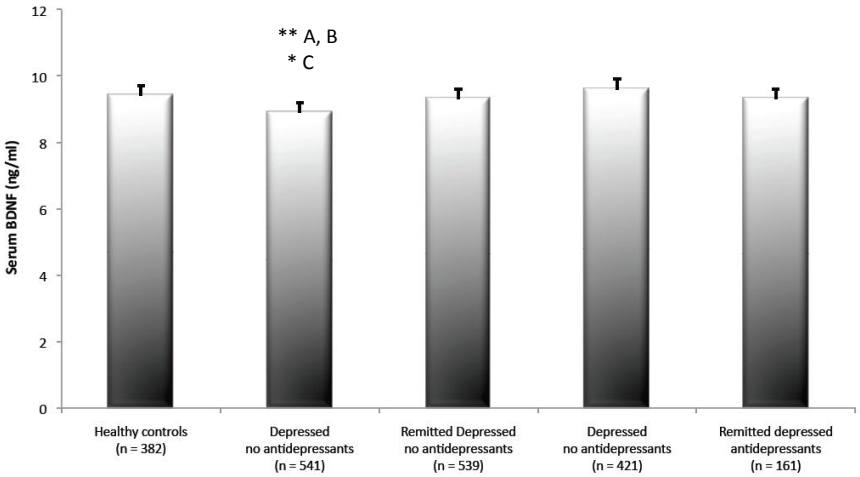

Figure 1. Plotted are mean serum BDNF concentrations by diagnosis and antidepressant status. Error bars reflect the *SEM*.

Serum BDNF concentrations are low in antidepressant-free depressed patients compared with controls (A: *d* = 0.19), antidepressant-free remitted persons (B: *d* = 0.15) and antidepressant-treated depressed patients (C: *d* = 0.23).

BDNF and the clinical features of depression

The exploration of the association between the clinical features of depression and serum BDNF was restricted to the antidepressant free currently depressed group, as BDNF concentrations in this group were low relative to controls. Pearson's correlation coefficients indicated that female gender and being in the early remission phase of depression (1–6 months) versus having a current episode were negatively associated with serum BDNF. Age, BMI, age at onset of depression and the presence of co-morbid anxiety were positively associated to serum BDNF (**Table 2 ↓**).

Basic covariates were entered in the first step of the multivariable regression analysis, followed by the clinical features that were entered in step two. Tolerance of the predictors was high (all > 0.70), indicating that our individual predictors were not redundant with one another. Error variances were normally distributed. Results of the first step showed that gender and age were significant predictors of BDNF concentrations. Women had lower concentrations of BDNF compared with men (ß = -0.10, *P* = 0.02) and older patients had higher concentrations of BDNF (ß = 0.11, *P* = 0.002) compared with younger patients. Results of the second step showed that none of the clinical features (listed in **Table 2 ↓**) was significantly associated with serum BDNF. Gender and age preserved its significance. BMI emerged as a significant (positive) predictor of serum BDNF. **Table 2 ↓** presents the results of the second step of the regression analysis.

BDNF and the use of antidepressants

A 2 (currently depressed versus depression in (full) remission) times 2 (antidepressant use; yes versus no) ANOVA showed that diagnostic status interacted with antidepressant use ($F_{1, 1578}$ = 4.19, *P* = 0.03), indicating that the use of an antidepressant during a depressive episode was associated with higher BDNF concentrations, whereas in the remission phase, the use of an antidepressant did not show such an association (see also **Figure 1 ↑**). Main effects of diagnostic status and antidepressant use were not observed. To uncover potential differences between various classes of antidepressants, we compared BDNF concentrations of depressed patients who used SSRIs, SNRIs, TCAs, NaSSAs or St John's wort among each other and those of antidepressant-free depressed patients. This analysis was restricted to the currently depressed group as the effect of the use of an antidepressant on serum BDNF concentrations was confined to this group. In this group, 67% (*n* = 282) used antidepressant for longer than 12 weeks. We observed a main effect of group ($F_{5, 941}$ = 4.29, *P* < 0.001). Post-hoc comparisons showed that, relative to not using an antidepressant, the use of SSRIs (*d* = 0.39) and St John's wort (*d* = 0.63) was associated with high

concentrations of BDNF. The use of a NaSSA was associated with low concentrations of BDNF relative to SSRI (*d* = 0.54) and St John's wort (*d* = 0.85) use. Analyses were run with and without co-varying for the severity of depressive symptoms and for the duration of antidepressant use. These analyses revealed a similar pattern of results. Furthermore, serum BDNF concentrations were unrelated to treatment duration (*r* = -0.02, *P* = 0.65), which might suggest that our findings were not driven by the duration of antidepressant use.

Table 2. Results of correlation and multivariable regression analyses of demographical and clinical characteristics with serum levels of BDNF in antidepressant free depressed patients (*n* = 541)

	r [A]	*B*	*95% CI B*	β	*P-value*
Gender (1 = male, 2 = female)	−0.13**	−0.65	−1.24 to −0.06	−0.10	0.03
Age (continuous, years)	0.17**	0.03	0.01 to 0.06	0.11	0.04
Education (continuous, years)	−0.04	−0.01	−0.09 to 0.08	−0.005	0.91
BMI (continuous)	0.13**	0.06	0.01 to 0.10	0.09	0.04
Physical activity (continuous per week)	−0.02	−0.001	−0.01 to 0.01	−0.009	0.83
Smoker (1=no, 2=yes)	−0.02	−0.07	−0.04 to 0.02	−0.02	0.66
Time of Blood withdrawal (continuous) [B]	−0.04	−0.004	−0.12 to 0.02	−0.04	0.23
Duration of serum storage (continuous)	0.02	0.14	−0.40 to 0.68	0.02	0.62
Current (= 1) vs. early remitted (= 2)[C]	−0.11*	−0.15	−0.50 to 0.25	−0.04	0.52
Severity (continuous)	0.03	−0.007	−0.04 to 0.02	−0.06	0.24
Single (= 1) vs. recurrent episode (= 2)	0.01	0.05	−0.56 to 0.66	0.007	0.88
Comorbid anxiety (1 = no, 2 = yes)	0.08*	0.31	−0.36 to 0.97	0.05	0.36
Age at onset (continuous)	0.14**	0.08	−0.04 to 0.18	0.07	0.21
Chronic depression (1 = no, 2 = yes)	0.07	0.19	−0.49 to 0.87	0.03	0.58
Suicide ideation (1 = no, 2 = yes)	0.06	0.59	−0.13 to 1.33	0.07	0.12

Abbreviations: BDNF, Brain-Derived Neurotrophic Factor; BMI, Body Mass Index; 95% *CI*, 95 percent *Confidence Interval*

[A] Univariate correlation with serum levels of BDNF; Pearson's *r* for continuous variables and Spearman's *ρ* for variables

[B] In minutes from 0600 hours

[C] The presence of a current (1 month) versus an early remission (1–6 months of remission) diagnosis

* Denotes statistical significance at *P* < .05

** Denotes statistical significance at *P* < .01

BDNF and the use of antidepressants

A 2 (currently depressed versus depression in (full) remission) times 2 (antidepressant use; yes versus no) ANOVA showed that diagnostic status interacted with antidepressant use ($F_{1, 1578}$ = 4.19, *P* = 0.03), indicating that the use of an antidepressant during a depressive episode was associated with higher BDNF concentrations, whereas in the remission phase, the use of an antidepressant did not show such an association (see also **Figure 1 ↑**). Main effects of diagnostic status and antidepressant use were not observed. To uncover potential differences between various classes of antidepressants, we compared BDNF concentrations of depressed patients who used SSRIs, SNRIs, TCAs, NaSSAs or St John's wort among each other and those of antidepressant-free depressed patients. This analysis was restricted to the currently depressed group as the effect of the use of an antidepressant on serum BDNF concentrations was confined to this group. In this group, 67% (*n* = 282) used antidepressant for longer than 12 weeks. We observed a main effect of group ($F_{5, 941}$ = 4.29, *P* < 0.001). Post-hoc comparisons showed that, relative to not using an antidepressant, the use of SSRIs (*d* = 0.39) and St John's wort (*d* = 0.63) was associated with high concentrations of BDNF. The use of a NaSSA was associated with low concentrations of BDNF relative to

SSRI (d = 0.54) and St John's wort (d = 0.85) use. Analyses were run with and without co-varying for the severity of depressive symptoms and for the duration of antidepressant use. These analyses revealed a similar pattern of results. Furthermore, serum BDNF concentrations were unrelated to treatment duration (r = -0.02, P = 0.65), which might suggest that our findings were not driven by the duration of antidepressant use.

DISCUSSION

Largely in accord with previous findings (Sen *et al*., 2008) and with the neurotrophin hypothesis of depression (Duman *et al*., 1997), our data showed that serum BDNF concentrations were low in antidepressant-free depressed patients compared with healthy controls. Our data further showed that BDNF concentrations were low in depressed patients who were not on antidepressant medication compared with antidepressant-free persons who were in full remission and that BDNF concentrations of this latter group were comparable to those of controls. Herewith, we establish as one of the first (Trajkovska *et al*., 2008) that low concentrations of BDNF in serum are a state characteristic of depression. In line with one study that reported low concentrations of BDNF in euthymic patients (Monteleone *et al*., 2008), we found that patients who were in early remission (1 – 6 months) had serum BDNF concentrations that were comparable to those of currently depressed patients. Thus, serum BDNF concentrations remain low after clinical improvement has set in. This could indicate that low concentrations of BDNF are a consequence of depressive symptoms that persist into early remission. Alternatively, the low concentrations of BDNF during early remission might also represent a scar of a depressive episode. These explanations could not be fully elucidated in the current study and longitudinal designs clearly need to be performed to understand this issue.

We were unable to replicate the earlier findings that a higher depression severity (Karege *et al*., 2002; Shimuzu *et al*., 2003) having a recurrent compared with a first episode of depression (Lee *et al*., 2007) and the occurrence of suicide ideation (Deveci *et al*., 2007; Kim *et al*., 2007) are accompanied by lower concentrations of BDNF. In fact, we even found that the early remission phase, which was accompanied by a lower symptom severity of depression (mean IDS scores were 22.4 ± 11.4 versus 32.4 ± 12.1 in early remitted and currently depressed patients respectively), was associated with somewhat lower BDNF concentrations compared with the current depressive state. The other clinical features (that is age at onset of depression, the presence of comorbid anxiety and the chronicity of depression) also were unrelated to serum BDNF in multivariable analyses. These findings, given the size of the current cohort, give us confidence in excluding the clinical features of depression as potential correlates of serum BDNF concentrations. This might be an important conclusion, as it hints that other (than specifically depression related) factors may be at play in the relative fall of BDNF concentrations during a depressive episode. Interestingly, being male and BMI were found to be positively associated with BDNF among antidepressant-free depressed patients. Although these findings were unsought, they parallel the results of some previous studies (Monteleone *et al*., 2005; Nakazato *et al*., 2003) and they give ground to interesting hypotheses. For example, as weight loss is a prime behavioral abnormality of depression (APA 1994) and often a residual symptom in early remission (Paykel 1985; Paykel *et al*., 1995) it could be that, alternations in BDNF concentrations are mediated by (transient) changes in eating behavior during, or in the aftermath of, a depressive episode. Likewise, weight gain is a documented side effect of antidepressant treatment (Kachur *et al*., 2005; Antilla and Leinonen, 2001) and thus the absence of weight loss could potentially explain the absence of a relative fall of BDNF in depressed patients during treatment with an antidepressant. Alternative factors that have been proposed to underlie the low concentrations of BDNF during depression are exposure to stressful life events. Two studies found that adverse life events are associated with lower

peripheral BDNF concentrations within a depressed and bipolar patient samples (Kauer-Sant'Anna *et al*., 2007; Grassi-Oliveira *et al*., 2008). Therefore, it seems worthwhile to integrate a wider range of variables, notably (early) adverse life events, but also genetic variants and their interactions with environmental variables (Gatt *et al*., 2009) in models that study the link between BDNF and depression.

In addition, we found that serum BDNF concentrations were higher in antidepressant-treated patients compared with patients who were antidepressant free. This finding largely is in accord with previous findings (Sen *et al*., 2008). We were able to expand previous findings by showing that the use of an antidepressant is associated with increased serum BDNF during a depressive episode but not during remission. This suggests that antidepressant-induced increases in BDNF occur in a disease state when BDNF functioning might be defective and not in remission when BDNF functioning is normalized. In addition, we found the increase in serum BDNF concentrations to be a specific associate of the use of SSRIs and St John's wort and not of the use of SNRIs, TCAs or NaSSAs. Although not directly confirmed, this finding might be explained by increased availability of extra-synaptic concentrations of serotonin. It is known that serotonin stimulates the expression of BDNF (Mattson *et al*., 2004; Martinowich and Lu, 2008). In line with this, we found the highest BDNF concentrations in patients who were treated with an agent that generally leads to an increase in the availability of serotonin, that is, SSRIs and St John's wort (Mann, 2005; Gaster and Holroyd, 2000). Furthermore, we found the lowest concentrations of BDNF in patients who were treated with agents that have little or no impact on the availability of serotonin, that is, NaSSAs (Kent, 2000; Antilla and Leinonen, 2001). Nevertheless, this antidepressant-specific finding seems at odds with the prediction of the neurotrophin hypothesis, stating that increases in BDNF concentrations are a key mediator for an antidepressant response to occur (Duman and Monteggia, 2006). According to this prediction, one might expect that antidepressants that are known to be about equally efficacious in the treatment of the symptoms of depression (Gaster and Holroyd, 2000; Kent 2000; Berton and Nestler, 2006) would have similar effects on serum BDNF concentrations. Yet another finding that seems hard to reconcile with the neurotrophin hypothesis is that the group of depressed persons who used antidepressants (for prolonged period and on a frequent base) had the highest BDNF concentrations, but also the highest symptom severity of depression. This suggests, to our belief that increases in peripheral BDNF concentrations do not parallel clinical effectiveness, or at least have no direct effects on depression characteristics such as its severity. Such a conclusion on the absence of direct effects could also be drawn on the findings that the severity of a depressive episode was unrelated to serum BDNF concentrations and that persons who were in early remission had similar concentrations of BDNF yet a marked lower depression severity as compared to currently depressed patients. Caution, however, is warranted when interpreting these findings because our patients were not randomly assigned to the various drugs (or no drug) conditions. Thus, our findings might be confounded by indication. An additional limitation of our study is that we relied on data that were collected in a single wave, precluding any form of causality. Furthermore, we measured serum concentrations of BDNF and assume that these measurements mirror the amount of BDNF in the brain. This assumption is validated on preclinical work that showed that cortical and peripheral concentrations of BDNF are correlated (Sartorius *et al*., 2009; Klein *et al*., 2010) but remains complicated, because in addition to neurons, several other tissues serve as sources of BDNF in serum (Karege *et al*., 2002). Various strengths of our study also seem evident and these include the use of multivariable techniques and the large sample size (that relates positive to all previous studies and to two previous meta-analyses (Brunoni *et al*., 2008).

In conclusion, we believe that our data indicate that low concentrations of BDNF in blood serum are a state characteristic of depression and thus an abnormality that is evident during the clinical state and the early remission phase of depression but not when the symptoms of depression are in full remission. Our findings further suggest that some of the core clinical features of depression are unrelated to serum

concentrations of BDNF. Finally, increases in serum concentrations of BDNF appear to be a specific pharmacological effect of a subset of antidepressants that does not parallel depression characteristics such as the severity of depression.

CHAPTER 5

Serum BDNF concentrations as peripheral manifestations of depression

Evidence from a systematic review and meta-analyses on 179 associations (N = 9,484)

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SIGNIFICANCE: This systematic review and meta-analyses is noteworthy in that it confirms that alternations in serum BDNF concentrations are peripheral manifestations of depresion. Yet, the evidence for this is way slimmer as was initially thought. An important implication of the message that this paper brings is that serum BDNF concentrations probably are of little clinical use.

ABSTRACT

Meta-analyses, published in 2008/2010, have confirmed abnormally low serum BDNF concentrations in depressed patients and normalisation of this by antidepressant treatment. These findings are believed to reflect peripheral manifestations of the *neurotrophin hypothesis*, which states that depression is secondary to an altered expression of BDNF in the brain. Since the publication of these meta-analyses, the field has seen a huge increase in studies on these topics. This motivated us to update the evidence on the aforementioned associations and, in addition, to compile the data on serum BDNF concentrations in relation to the symptom severity of depression. Using a manifold of data as compared to earlier meta-analyses we find low serum BDNF concentrations in 2,384 antidepressant-free depressed patients relative to 2,982 healthy controls and to 1,249 antidepressant-treated depressed patients (Cohen's *d* = -0.71 and -0.56, *P*-values < .0000001). When publication bias is accounted for, these effect-sizes become substantially smaller (*d* = -0.47 and -0.34 respectively, *P*-values < .0001). We detect between-study heterogeneity in outcomes for which only year of publication and sample size are significant moderators, with more recent papers and larger samples sizes in general being associated with smaller between-group differences. Finally, the aggregated data negates consistent associations between serum BDNF concentrations and the symptom severity of depression. Our findings corroborate the claim that altered serum BDNF concentrations are peripheral manifestations of depression. However, here we highlight that the evidence for this claim is slimmer as was initially thought and amidst a lot of noise.

INTRODUCTION

The *neurotrophin hypothesis*, originally formulated in 1997 by Duman, Heninger and Nestler, characterizes major depressive disorder as being secondary to aberrant neurogenesis in brain regions that regulate emotion and memory. According to this hypothesis; aberrant neurogenesis is brought about by a (stress induced) lower expression of Brain-derived Neurotrophic Factor (BDNF). In addition, the neurotrophin hypothesis predicts that antidepressants are efficacious because they increase BDNF expression and herewith resolve aberrant neuronal plasticity (Duman and Monteggia, 2006; Park and Poo, 2013). A large pre-clinical literature, allowing for mechanistic insights, fits very well with these predictions. Taliaz and colleagues (2010) for instance, showed in rats that a reduction of BDNF in the dentate gyrus impairs neurogenesis and induces depressive-like behavior. Human post-mortem studies have indicated similar alternations in the brains of persons who were depressed at the time of dying (Thompson Ray et al., 2011). Further support for abnormalities in BDNF expression in depressed patients comes from clinical studies. Karege *et al.* (2002) as the first, found serum BDNF concentrations to be low in depressed patients as compared to healthy controls and lowest in persons with the highest levels of symptom severity. Shimuzu *et al.* (2003) were the first to show an increase in serum BDNF concentrations in the course of antidepressant treatment.

These findings generated a buzz of research activity and in 2008/2010 the clinical data were summarized in three meta-analyses (Sen *et al.,* 2008; Brunoni *et al.,* 2008; Bocchio-Chiavetto *et al.,* 2010). These meta-analyses, basically including the same 11 studies (N ~ 968) confirmed the finding of low serum BDNF concentrations in untreated depressed patients (effect size [Cohen's d] ~ -1) and normalization of this by antidepressant treatment (d ~ 1) whilst suggesting that these associations were not hampered by between-study heterogeneity or publication bias. Accordingly, the conclusion was: *BDNF may have potential use as biomarker for psychiatric disorders or as a predictor of antidepressant efficacy* (Sen *et al.,* 2008; page 527). Since then, the field has seen an abundance of new data on these topics. Important is that this new data entails striking variation in outcomes across studies (see for instance Basterzi *et al.,* 2009 or Elfing *et al.,* 2012). This, and the abundance of new data, motivated us to update the current state of knowledge by calculating pooled effect-size estimates on differences in serum BDNF concentrations among:

- Antidepressant-free depressed patients and healthy controls subjects
- Antidepressant-free- and antidepressant-treated depressed patients
- Antidepressant-treated depressed patients and healthy controls subjects

An additional aim was to compile the data on the putative relation between serum BDNF concentrations and the symptom severity of depression in:

- Antidepressant-free depressed patients
- Antidepressant-treated depressed patients
- Healthy control subjects

A final aim, made possible by a large amount of studies, was to learn on the potential influence that some relevant moderators might have on the outcomes of our interest.

Method

We adhered to the guidelines that are recommended by the preferred reporting items for systematic reviews and meta-analyses statement (Moher *et al.,* 2009). The literature search, decisions on inclusion, data extraction, and quality control were all performed independently by ≥ two of the authors.

Search Strategy

We searched the PUBMED, Embase, and PsychInfo through April 1st 2013 to identify eligible human studies on serum BDNF concentrations in healthy controls, depressed patients or in both. These digital searches were supplemented by backward searches in which the references to the seminal papers of interest were screened (Karege *et al.,* 2002; Shimuzu *et al.,* 2003) and by examining the reference sections of the retrieved papers.

We included peer-reviewed human studies that reported data on serum BDNF concentrations in healthy controls, and antidepressant-free and treated depressed patients. Inclusion was independent of clinical- and the methodological characteristics of the sample or study. Non-empirical studies were excluded, as were studies that were not written in English, Dutch, German or Spanish. Papers that reported on overlapping samples were excluded except for the one that reported on the largest number of subjects.

Data Extraction

We extracted, as primary outcomes, mean serum BDNF concentrations and Standard Deviation (*SD*) as a function of diagnostic status and antidepressant use and/or indices on the relation between BDNF concentrations and the symptom severity of depression (*e.g.,* Pearson's *r*). When BDNF concentrations were assessed at multiple time points we extracted the data recorded at baseline and at the longest follow-up period.

We also extracted data on mean age, gender distribution, depression severity, antidepressant use (subdivided by SSRIs, TCAs, and SNRIs), duration of antidepressant use, and the number of subjects in the study. Where records did not provide sufficient information, corresponding authors were contacted and the required data was requested. In those cases where non-significant results were reported in a paper (*e.g., P* > .05) and authors did not reply to our request, we assigned an estimated effect-size of zero.

Quality Assessment

We used the Newcastle-Ottawa Scale (NOS; Wells *et al.,* 2013) to assess the quality of the included studies. Overall quality score was defined as the frequency of criteria that were met by the particular study. We excluded NOS items 4 and 7 because these are meaningless in the context of the current paper. Mean-quality score of the included studies was 3.18 (Standard Deviation [*SD*] = 0.14). The agreement between the independent raters was excellent (Cohen's Kappa = 0.89, Standard Error [*SE*] = 0.03).

Statistical analysis

All calculations were performed using comprehensive meta-analyses 2.0 (Borenstein *et al.,* 2009). Random effects models were applied to calculate pooled Cohen's *d* (Cohen 1988) on between-group differences in serum BDNF concentrations. Pooled correlation coefficients were calculated on the relation between serum BDNF concentrations and the symptom severity of depression. All outcomes were weighted using inverse variance methods (Mosteller and Golditz, 1996). Statistical significance of the pooled effect-sizes was assessed using a Confidence Interval (*CI*) of 95%. The I^2 measure was used to quantity the amount of between-study heterogeneity and considered to be high when I^2 > 50% (Higgins and Thompson, 2002). Statistical significance of heterogeneity was assessed using the *Q*-statistic (Borenstein *et al.,* 2009).

Through meta-regression analyses the possible moderating effects of between-study differences on outcomes was evaluated. We considered the number of subjects included in the study, year of publication, mean age, symptom severity of depression of the patient sample, gender distribution, and the NOS score as potential moderators for all outcomes of interest. The severity rating scales that were used differed

between studies. These instruments use different values to quantify severity (*e.g.,* Hamilton 1960 or Rush *et al.,* 1996) that do not necessarily equate to each other. Therefore, we used the validated severity categories: none, mild, moderate, severe, and very severe that can be derived from the continuous scores on each of these instruments as potential moderating variable. The moderation analysis on the difference in serum BDNF concentrations between healthy controls and antidepressant-treated depressed patients in addition included variables coding for the class of antidepressant and the duration of treatment. For the meta-analysis on antidepressant-free and treated depressed patients, the set of moderators was extended with a variable coding for change in depression severity over treatment defined as the percentage of improvement on the depression rating scale that was used.

Publication bias was assessed by inspection of funnel-plots and the Egger test (Egger et al., 1997). The trim-and-fill procedure, a validated manner to estimate an effect-size after bias has been taken into account (Duval and Tweedie, 2000; Peters *et al.,* 2007), was performed in case of publication bias. Power and sample size calculations were performed using G*Power (Faul *et al.,* 2009). Stability of our results was evaluated by sensitivity analyses in which each study was excluded from analyses at a time.

Results

Our initial search generated 730 papers of which 55 fulfilled the inclusion criteria for at least one of our meta-analyses. From these papers we could extract 124 between-group effect-size estimates and 55 correlation coefficients. For details on the search strategy we refer to the flow chart (**Figure 1 ↓**). **Table 1 ↓** lists in which meta-analysis the papers were included and provides demographic and clinical characteristics of the included studies.

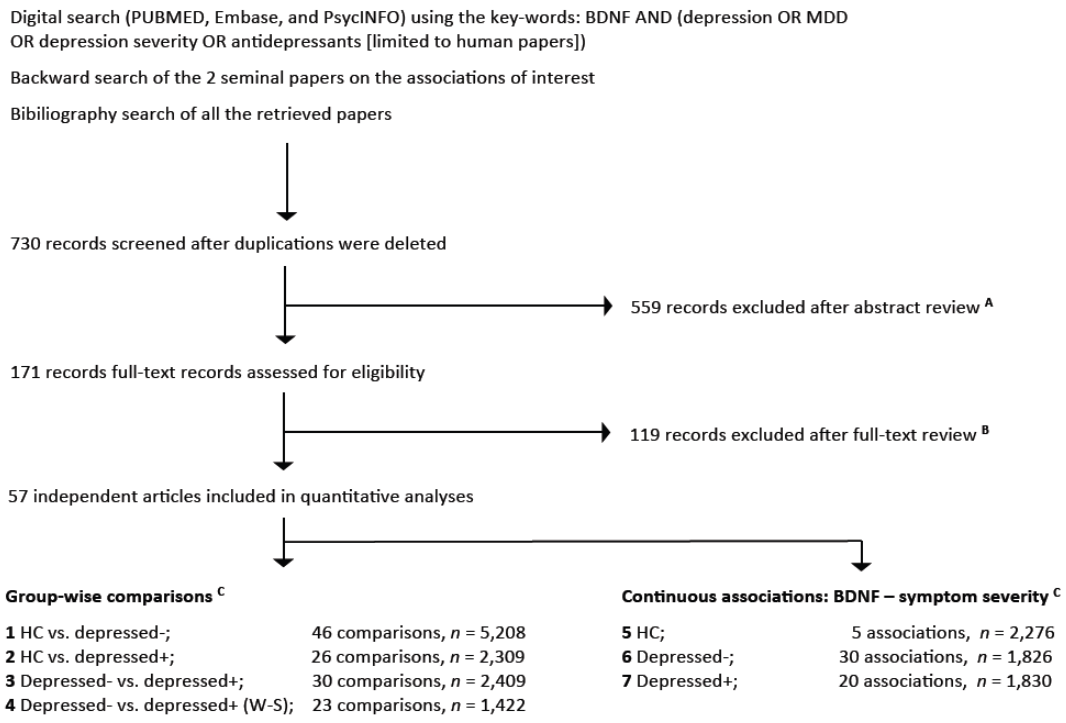

Figure 1. Flow-chart of the search strategy and results

Abbreviations: BDNF; Brain-Derived Neurotrophic Factor; HC; Healthy Controls, MDD; Major Depressive Disorder.

[A] 192 records reported on the BDNF gene, 193 records were reviews, perspectives, comments or hypotheses, 36 records reported on animal data, 14 records were post-mortem studies, 12 records were in vitro studies, and 111 records did not rapport on BDNF.

[B] 2 records reported overlapping data, 3 records reported on the BDNF gene, 64 records reported on plasma BDNF concentrations, 3 records were reviews, 43 records did not reported on serum BDNF concentrations in illnesses other than depression and did not indicate that depression related assessments were performed.

[C] Most of the articles provided input for > 1 meta-analytical effect-size. The numbersof comparisons/associations therefore do not add up to 57.

Table 1. Summary of study characteristics of included studies (studies are sorted by year and month of publication)

Author, year	meta-analysis [A]	design [B]	*N*	% female	mean age	patient status	*n* [C]	severity
Karege *et al.,* 2002	(1)(5)	B-S	60	50	37	HC	30	MADRS
						Depressed +	30	
Shimuzu *et al.,* 2003	(1)(2)(3)(5)(6)	both	83	43	43	HC	50	HAMD
						Depressed -	16	
						Depressed +	17	
Gervasoni *et al.,* 2005	(1)(2)(3)(4)	both	52	54	40	HC	26	MADRS
						Depressed -	26	
						Depressed +	26	
Gonul *et al.,* 2005	(1)(2)(3)(4)(5)	both	46	71	36	HC	18	HAMD
						Depressed -	28	
						Depressed +	28	
Karege *et al.,* 2005	(1)(4)	B-S	78	56	34	HC	35	MADRS
						Depressed -	43	
Aydemir *et al.,* 2005	(1)(2)(3)	both	20	80	36	HC	10	HAMD
						Depressed -	10	
						Depressed +	10	
Zanardini *et al.,* 2006	(6)	W-S	16	69	56	Depressed +	16	HAMD
Lommatzsch *et al.,* 2006	(1)(5)	B-S	80	100	28	HC	62	EPDS
						Depressed -	18	
Ayedemir *et al.,* 2006	(1)(2)(3)	both	40	100	35	HC	20	HAMD
						Depressed -	20	
						Depressed +	20	
Bocchi-Chiavetto *et al.,* 2006	(6)	W-S	12	70	53	Depressed +	12	MADRS
Lang *et al.,* 2006	(4)	B-S	24	*NK*	46	Depressed -	8	MADRS
						Depressed +	16	
Aydemir *et al.,* 2007	(1)	B-S	50	74	33	HC	26	HAMD
						Depressed-	24	
Yoshimura *et al.,* 2007	(1)(2)(3)(4)	both	72	65	46	HC	30	HAMD
						Depressed -	42	
						Depressed +	42	
Ziegenhorn *et al.,* 2007	(1)(5)	B-S	465	48	85	HC	259	HAMD
						Depressed-	91	
Hellweg *et al.,* 2007	(3)	W-S	40	71	51	Depressed -	40	HAMD
						Depressed +	40	
Okamoto *et al.,* 2008	(6)	B-S	18	50	61	Depressed +	18	HAMD
Stanek *et al.,* 2008	(4)	B-S	34	56	73	HC	34	PRIME
Huang *et al.,* 2008	(1)(2)(3)	both	218	72	33	HC	107	HAMD
						Depressed -	111	
						Depressed +	79	
Piccini *et al.,* 2008	(1)(2)(3)	both	30	83	42	HC	15	HAMD
						Depressed -	15	
						Depressed +	15	
Matrisciano *et al.,* 2009	(1)(2)(3)	both	41	51	37	HC	20	HDRS
						Depressed -	21	
						Depressed +	21	
Basterzi *et al.,* 2009	(1)(2)(3)	both	58	67	33	HC	15	HAMD
						Depressed -	43	
						Depressed +	43	
Gorgulu *et al.,* 2009	(1)(2)(3)	both	72	69	36	HC	31	HAMD
						Depressed -	41	
						Depressed +	22	
Grønli *et al.,* 2009	(6)	B-S	15	60	70	Depressed +	15	HAMD
Umene-Nakano *et al.,* 2009	(1)(5)	B-S	40	25	44	HC	20	HAMD
						Depressed -	20	
Fernandes *et al.,* 2009	(2)(6)	B-S	40	60	42	HC	30	HAMD
						Depressed +	10	
Lee *et al.,* 2009	(1)	B-S	132	61	74	HC	98	GDS
						Depressed -	34	
Ozan *et al.,* 2010	(1)	B-S	122	70	34	HC	56	HAMD
						Depressed -	66	
Diniz *et al.,* 2010	(1)(4)	B-S	71	83	70	HC	42	HAMD
						Depressed -	29	
Eker *et al.,* 2010	(1)(4)	B-S	47	75	31	HC	22	HAMD
						Depressed -	25	
Bocchi-Chiavetto *et al.,* 2010	(1)(4)	B-S	84	81	43	HC	59	MADRS
						Depressed -	25	

Table 1 *continues on the next page*

Table 1 *continued*

Author, year	meta-analysis [1]	design [2]	*N*	% female	mean age	patient status	*n*	severity
Hu	(1)	B-S	84	73	43	HC	28	HAMD
						Depressed a -	28	
						Depressed b -	28	
Zhou *et al.,* 2011	(1)	B-S	123	*NK*	*NK*	HCa	30	HAMD
						HCb	58	
						Depressed -	35	
Su *et al.,* 2011	(1)	B-S	52	0	23	HC	21	*NK*
						Depressed -	31	
Rojas *et al.,* 2011	(3)	B-S	34	71	42	Depressed -	34	HAMD
						Depressed +	34	
Yoshimura *et al.,* 2011	(3)(4)	W-S	132	60	51	Depressed –	132	HAMD
						Depressed +	132	
Wolkowitz *et al.,* 2011	(1)(2)(3)	B-S	57	36	39	HC	28	HAMD
						Depressed -	29	
						Depressed +	25	
Kobayakawa *et al.,* 2011	(1)	B-S	162	30	65	HC	81	HADS
						Depressed -	81	
Terraciano *et al.,* 2011	(5)	B-S	2,099	62	51	HC	1,661	CES-D
						Depressed -	438	
Molendijk *et al.,* 2011	(1)(2)(3)(4)(5)(6)	B-S	1,344	65	42	HC	382	IDS
						Depressed -	541	
						Depressed +	421	
Toups *et al.,* 2011	(6)	B-S	70	80	47	Depressed +	70	HAMD
Satomura *et al.,* 2011	(2)(4)(5)	B-S	272	63	53	HC	163	HAMD
						Depressed +	109	
Sasaki *et al.,* 2011	(1)(2)(3)(5)(6)	B-S	52	56	13	HC	22	CDRS-R
						Depressed -	19	
						Depressed +	11	
Sozeri-Varma *et al.,* 2011	(1)(4)	B-S	70	73	37	HC	40	HAMD
						Depressed -	30	
Bus *et al.,* 2012	(4)	B-S	1,230	50	61	HC	1,230	BDI
Gedge *et al.,* 2012	(5)	W-S	29	69	45	Depressed +	29	HAMD
Gazal *et al.,* 2012	(1)	B-S	72	100	25	HC	36	BDI
						Depressed -	36	
Birkenhäger et al., 2012	(6)	W-S	42	43	47	Depressed -	42	HAMD
Deuschle *et al.,* 2012	(1)(2)(3)(4)	W-S	70	72	52	HC	14	HAMD
						Depressed -	56	
						Depressed +	56	
Harvey *et al.,* 2012	(1)(5)	W-S	200	49	44	HC	89	PHQ-9
						Depressed -	111	
Oral *et al.,* 2012	(1)(5)	B-S	79	68	27	HC	40	BDI
						Depressed -	39	
Karlovic *et al.,* 2012	(1)	B-S	264	50	46	HC	142	HAMD
						Depressed -	122	
Jeon *et al.,* 2012	(1)(2)(3)(4)	W-S	155	71	44	HC	50	HAMD
						Depressed -	105	
						Depressed +	105	
Yoshida *et al.,* 2012	(2)(5)	B-S	147	56	38	HC	78	SIGH-D
						Depressed +	69	
Elfving *et al.,* 2012	(1)(2)	B-S	406	81	46	HC	289	ICD-10
						Depressed -	117	
						Depressed +	45	
Papakostas *et al.,* 2013	(1)	B-S	79	52	36	HC	43	HAMD
						Depressed -	36	

Abbreviations: HC, Healthy controls; Depressed -, antidepressant free; Depressed +, antidepressant treated; *NK*, Not Known.

[A] This column indicates in which meta-analysis the study that is indicated in the corresponding row is included: (1) HCs vs. depressed -; (2) HCs vs. depressed +; (3) Depressed - patients vs. MDD+; (4-6) regard meta-analyses on continuous associations between serum BDNF concentrations and depression severity scores: (4) in HC's; (5) in depressed -; (6) in depressed +.

[B] This column, design, indicates whether Within-Subjects data (W-S), a Between-Subjects data (B-S), or a combination of these types of data (both) is used by the study that is indicated in the corresponding row.

[C] Note that the numbers in the column *n* do not add to the numbers as they are given in the column *N*. This is because the numbers in column *n*, in some instances, are counted double (*e.g.,* before and after antidepressant treatment in longitudinal designs).

Meta-analyses

Random-effects meta-analyses showed that antidepressant-free depressed patients had lower BDNF concentrations as compared to healthy controls (*d* = -0.71, 95% *CI* = -0.89 — -0.53*, P* < .0000001; 46 comparisons, *n* = 5,203; see **Figure 2 ↓**) and to those of antidepressant-treated depressed patients (*d* = -0.56, 95% *CI* = -0.77 — -0.35*, P* < .00001, 28 comparisons, *n* = 4,204). Repeating this latter analysis using only studies that reported pre- and post-treatment BDNF concentrations gave a somewhat higher effect-

size estimate (*d* = -0.74, 95% *CI* = -1.04 — -0.45*, P* < .0000001, 23 comparisons, within-subjects data on 711 patients pre- and post-treatment). Differences in BDNF concentrations among healthy controls and antidepressant-treated depressed patients were not observed (*d* = 0.07 *P* = .52; 24 comparisons, *n* = 3,720). Forest plots (except **Figure 2 ↓**) are provided as supplementary materials (**Figure S1**–**S3**) in **Appendix III** of this thesis.

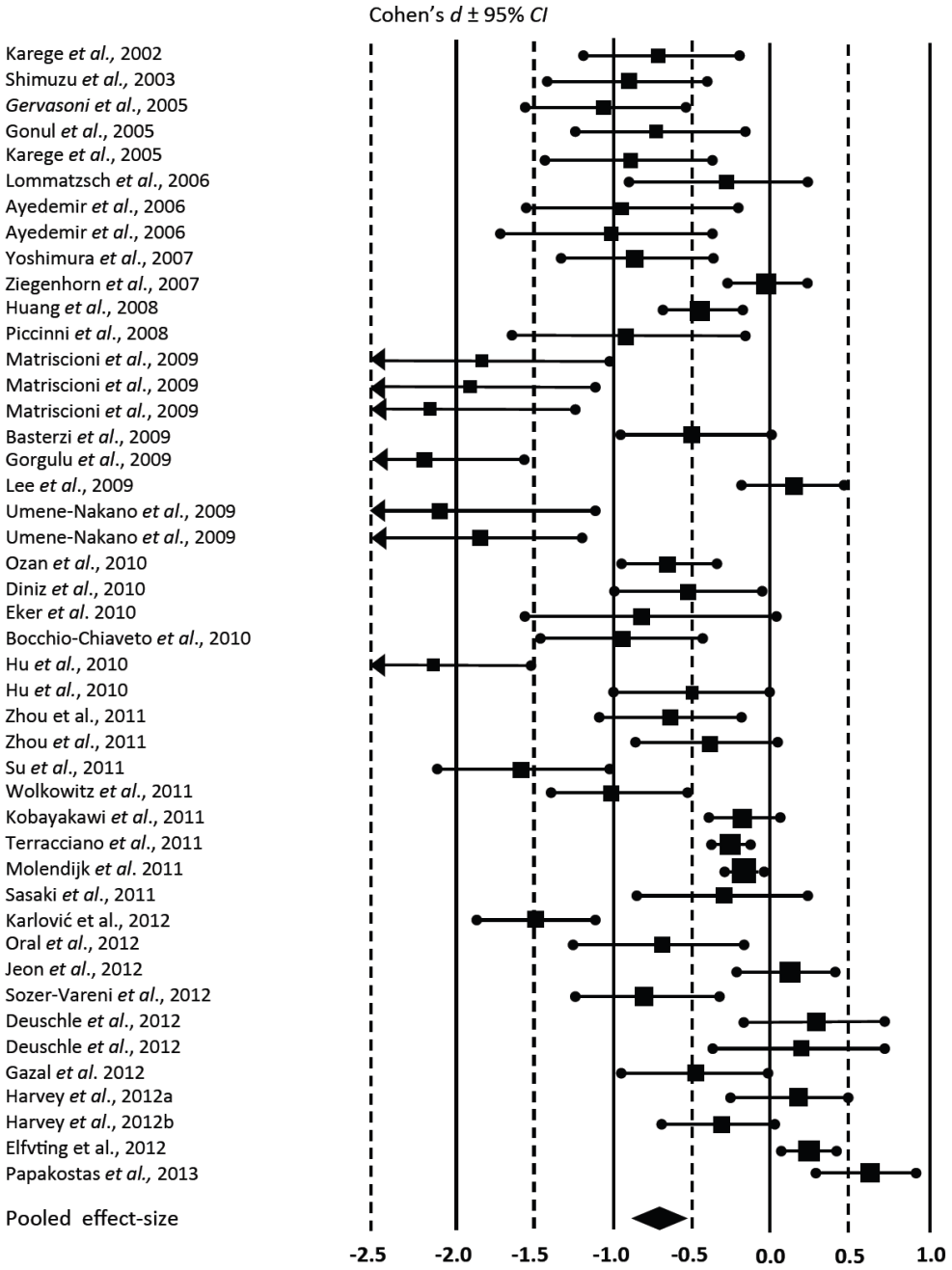

Figure 2. Forrest plots for random effect meta-analyss on differences in serum BDNF concentrations between healthy control subjects and antidepressant-free depressed patients. The sizes of the squares are proportional to sample size.

A meta-analysis aggregating 30 associations (*n* = 1,807) on the relation between BDNF concentrations and the symptom severity of depression in antidepressant-free depressed patients yielded a statistically significant, negative correlation (*r* = -0.19; 95% *CI* = -0.28 — -0.10*, P* < .00001). There was no evidence for a relation between serum BDNF concentrations and depression severity in antidepressant-treated depressed patients (*r* = -0.02; *P* = .36, 20 associations, *n* = 1,820) or in healthy controls (*r* = -0.02; *P* = .41, 5

associations, *n* = 2,276). Forest plots are provided as supplement (**Figure S4–S6**) in **Appendix III** of this thesis.

Between–study heterogeneity and moderation analyses

A large amount of between-study heterogeneity in outcomes was identified in all meta-analyses that yielded significant outcomes (55% < I^2 < 87%, for I^2-, *Q*-, and *P*-values we refer to **Table 2 ↓**).

Table 2. Statistics on between study heterogeneity and publication bias for the meta-analysis indicated in the row

	No. of associations	No. of subjects			Heterogeneity			Publication bias	
		HC	Depressed-	Depressed+	I^2	*Q*	*P*	Egger's *t*	*P*
Group-wise comparisons									
HC vs depressed -	41	2,911	2,292	*NA*	86.1%	287.6	< .001	4.2	< .001
HC vs depressed+	24	2,591	*NA*	1,129	84.6%	150.2	< .001	1.4	.16
Depressed- vs depressed +	27	*NA*	2,955	1,249	84.4%	165.1	< .001	2.5	< .05
Depressed- vs depressed + W-S [1]	23	*NA*	711	711	83.9%	136.8	< .001	2.6	< .05
Continuous associations									
HC	5	2.276	*NA*	*NA*	14.8%	4.7	.32	1.0	.15
Depressed -	29	*NA*	1,807	*NA*	67.9%	87.2	< .001	2.5	< .05
Depressed+	19	*NA*	*NA*	1,820	18.3%	48.9	.36	0.6	.53

Abbreviations: HC, Healthy controls; depressed-, antidepressant free; depressed+, antidepressant treated; *NA*, Not Applicable; W-S, Within-Subjects data

[1] Here, only associations were included that were derived using a within-subjects designs (*i.e.,* treatment studies)

In a series of meta-regression analyses, we aimed to identify sources of heterogeneity in outcomes. We observed that differences in serum BDNF concentrations among antidepressant-free depressed patients and healthy control subjects could partly be explained by sample size ($r = -0.33$, $R^2 = 0.11$, $P = 0.03$) and by year of publication ($r = -0.30$, $R^2 = 0.09$, $P = 0.04$), with larger samples and more recently reported papers in general reporting smaller between-group differences. In the meta-analysis on changes in serum BDNF concentration over the course of antidepressant treatment, we found that a larger decrease in symptom alleviation was accompanied by a larger increase in BDNF concentrations ($r = -0.48$, $R^2 = 0.22$, $P = 0.01$). Other moderators, including NOS score, were not observed (see **Table 3 ↓** for all coefficients). Moderation analyses were not performed when between-study heterogeneity was not detected.

Publication bias and power

Visual inspection of the funnel plots suggested that there was evidence for publication bias in all meta-analyses that yielded a significant outcome. Egger's tests confirmed this (*t*-values in the range 2.5 – 4.2, *P*-values all < .05, see **Table 2 ↑** for exact values).

Trim-and-fill estimations were used to assess the impact of publication bias. The meta-analysis on differences in BDNF concentrations among healthy controls and untreated depressed patients suggested that 9 studies had to be imputed to result in a symetric funnel plot. Imputation led to a smaller, yet significant, effect-size (*d* = -0.47, 95% *CI* = -0.64 — -0.27, *P* < .000001). The pattern of publication bias was similar in the meta-analyses comparing group differences among antidepressant-free and treated subjects, where 5 (all data) and 4 studies (within-subjects data) needed to be imputed to yield a symetric funnel plot. Also here, imputation led to smaller effect-size estimates (*d* = -0.54 and -0.34 respectively, *P*-values < .001). Likewise, for the meta-analyses on the continous association between serum BDNF concentrations and the symptom severity of depression in untreated depressed persons, the trim-and-fill estimations suggested that 5 studies had to be imputed to result in a symetric funnel plot pattern. Herewith, the effect-size estimate (*r* = -0.07) was no longer statistically signinicant. Funnel plots are provided in **Appendix III** (**Figures S7–S10**).

Table 3. Associations (Pearson's correlation coefficients for continuous- and Spearman's Rho correlation coefficients for categorical variables) between study characteristics and study effect size (by meta-analysis)

Group differences	HC vs. depressed- 41 effect-sizes *n* = 5,203	HC vs. depressed+ 24 effect-sizes *n* = 3,720	Depressed- vs. depressed+ 27 effect-sizes *n* = 4,204	Depressed- vs. depressed+ W-S 23 effect-sizes *n* = 1,422
Gender (percentage female)	0.16	0.11	0.06	0.08
Age (mean, years)	0.13	-0.11	0.08	0.11
Depression severity (cat.)	-0.17	-0.10	-0.21	-0.07
Percentage SSRI	*NA*	0.29	-0.35 #	-0.34
Percentage TCA	*NA*	-0.21	0.13	0.11
Percentage SNRI	*NA*	-0.10	0.17	0.17
Percentage NaSSA	*NA*	-0.14	0.14	0.15
Duration of treatment (weeks)	*NA*	-0.34	0.04	0.04
Clinical response on treatment	*NA*	*NA*	*NA*	-0.48 *
Sample size (*n*)	0.33 *	-0.15	0.25	0.21
Year of publication	0.30 *	-0.16	0.18	0.18
Study quality (criteria met)	0.04	0.06	0.35 #	0.34

Abbreviations: HC, Healthy controls; depressed-, antidepressant free; depressed+, antidepressant treated; *NK*, Not Known; W-S, Within-Subjects data only (*i.e.,* associations were that were derived using a within-subjects design.
[1] Given that there was no evidence for between-study heterogeneity, moderation analysis was not performed in these sub-groups.
* Statistically significant at $P < .05$ # Trend-like finding at $P < .10$

We calculated the numbers of subjects that are needed to detect differences with a power of 0.80 at an α-level of .05 (one-sided). Hereto we used the pooled effect-size estimates that were corrected for publication bias. These calculations suggested that 57 subjects in each group would be neccesary to reliably detect differences in serum BDNF concentrations between healthy controls and antidepressant free depressed subjects. For differences in serum BDNF concentrations among antidepressant-free and treated persons, this number would be 108. Based on this, the majority of the included samples was not sufficiently powered (observed median sample size = 36). Sample-size calculations were not performed for continuous associations between serum BDNF concentrations and the symptom severity of depression since these were not statistically significant.

Sensitivity analyses indicated that none of the study findings was unduly driven by the effect of a particular study.

Discussion

Here we confirm, based on a manifold of data as compared to previous meta-analyses (Sen *et al.,* 2008; Brunoni *et al.,* 2008; Bocchio-Chiavetto *et al.,* 2010) that serum BDNF concentrations are low in untreated depressed patients and normalized by antidepressant treatment. The moderate to large effect-sizes that we rapport on these differences (random-effects meta-analyses, *d* = -0.71 and -0.56 respectively) are similar to the ones that were reported in the seminal studies (Karege *et al.,* 2002; Shimuzu *et al.,* 2003) and in previous meta-analyses. These findings are not new. The novelty of our work, instead is that our analyses highlight a large amount of unexplained between-study heterogeneity in outcomes and publication bias that together may call for a critical interpretation of the claim that altered serum BDNF concentrations are related to, and a clinical useful marker for, the illness depression.

We find a large amount of between-study heterogeneity in outcomes and none of the theoretically relevant variables that we tested (*e.g.,* the symptom severity of depression or gender distribution of the sample) was associated with this. Understanding the sources of the observed heterogeneity is essential and obviously, it may have come from between-sample characteristics that were not tested in our study, such as alcohol consumption and smoking (Bus *et al.,* 2011), sleep problems (Giese *et al.,* 2013), seasonality (Molendijk *et al.,* 2012), or exposure to trauma (Elzinga *et al.,* 2011). Given that depression is a

heterogeneous illness (Rush 2007), heterogeneity in outcomes may also have come from diversity in clinical characteristics of patient samples. The severity of depression, however, did not explain it. Unfortunately, we did not have the opportunity to test many of the other clinical characteristics because most of the included studies did not report on these variables.

We did find an artificial base for the heterogeneity in outcomes. First, a large part of the studies included in our meta-analysis was underpowered. Given that a low level of power increases the false versus true positive ratio (Sterne and Smith, 2001), some overly positive findings may have been among the studies that we included, causing heterogeneity in outcomes. Second, we found that sample size and year of publication were significant predictors of between-study heterogeneity, with larger samples and more recently published findings being associated with smaller between-group differences. This indicates publication bias; a particular threat to the validity of a meta-analysis (Dickersin 1990). We indeed found evidence for publication bias in funnel-plots (Egger *et al.,* 1997) and we applied validated trim-and-fill procedures to provide effect-size estimates that account for this (Peters *et al.,* 2007). These yielded attenuated effect-size estimates that were about half as large as those reported in previous meta-analysis (Sen *et al.,* 2008; Bocchio-Chiavetto *et al.,* 2010) and of moderate magnitude at best (*d* = -0.47 through -0.34). The often discussed association between serum BDNF concentrations and the symptom severity of depression (*e.g.,* Karege et al., 2002), for which we initially found some evidence, even lost its statistical significance after correcting for publication bias and thus likely does not exist. Given that the relevance of a diagnostic biomarker (*i.e.,* a variable that is able to distinguish between diagnostic groups; Kapur *et al.,* 2012) depends on the magnitude of an effect-size (and not on statistical significance *per se*; Kapur *et al.,* 2012), we conclude that serum BDNF concentrations are likely to be of little clinical use (as has been suggested in two earlier excellent reviews Groves 2007; Gass and Hellweg, 2010). Complicating this even more is that low serum BDNF concentrations have been reported in persons diagnosed with schizophrenia (Green *et al.,* 2011), bipolar disorder (Fernandes *et al.,* 2011), eating disorders (Montleone *et al.,* 2005), and anxiety (Molendijk *et al.,* 2012) indicating that serum BDNF concentrations are not specific enough to differentiate among diagnoses. Multiple-assay methods may serve a role as biomarker better, as recently has been shown (Papakostas *et al.,* 2013).

Although limited in scope with regard to clinical utility, our findings do not dismiss the possibility that abnormalities in BDNF expression reflect the pathophysiological processes that may underlie depressive illnesses (Duman *et al.,* 1997; Duman and Monteggia, 2006). Even more, the associations that we report on, also when adjusting for publication bias, stand out as being strong when compared to other biological abnormalities in depression, for instance blood markers for immune dysregulation (*e.g.,* CRP and IL-6 [*d* = 0.15 and 0.25 respectively]) or HPA-axis activity (*e.g.,* adrenocorticotropin hormone [*d* = 0.28] for a review on these abnormalities see Penninx *et al.,* 2013).

A difficulty that remains however is that we studied peripheral BDNF concentrations. There are indications that BDNF concentrations measured in serum reflect BDNF activity in the brain (*e.g.,* Dawood *et al.,* 2007; Klein *et al.,* 2010). However, it has never been proven that peripheral BDNF concentrations directly reflect or influence the pathophysiology of depression. A complication is that other tissues than the brain, including immune-, liver-, smooth muscle-, and vascular endothelial cells serve as sources of BDNF (Cassiman *et al.,* 2001; Karege *et al.,* 2002b). The lower peripheral BDNF concentrations in depression and up-regulation of this in the course of antidepressant treatment therefore may be an epiphenomenon resulting from an altered BDNF expression (or metabolism) by these peripheral organs. Therfore, the alternations that we rapport on do not neccesarly indicate that similar alternations occur at a central level and conclusions should not be overbearing.

Strengths and limitations

The work presented herein has as obvious strength that it is based on a large amount of data (total *N* = 9,484), yielding in general accurate effect-size estimates (Ioannidis 2005). Another strength is that through sensitivity- and moderation analyses we addressed the potential influence of single studies and sources of heterogeneity. Notwithstanding this, our work carries limitations that need to be reflected upon.

Some limitations regard the methods that we used. First, we relied on funnel-plot assymetry and trim-and-fill estimations to assess publication bias. These methods are limited in that one never knows whether asymmetry in a funnel-plot is due to publication bias or to unmeasured differences between studies (Munafo and Flint, 2004) and whether the most extreme effect-sizes are the ones that are left unpublished (Peters *et al.,* 2007). Second, in at least some regards the methods that we used were limited with regard to their ability to detect associations. The meta-regression analyses, for instance, may have been underpowered. Besides, *P*-values were not adjusted for multiple comparisons. Also important is that there may have been noise in our assessment of individual study quality. The NOS scale that we used to this end, although recommended by the Cochrane Collaboration (www.cochrane.org) is not rigorously validated and therefore our quality assessments may have been unreliable (Sanderson *et al.,* 2007). Together, this may have limited our ability to detect true associations (*i.e.,* false negatives) or may have led to the detection of associations that in reality do not exist (*i.e.,* false positives). Finally, our findings are limited in scope in that they cannot be directly generalized to other BDNF parameters such as plasma or whole blood BDNF concentrations since there is no one–to–one relationship among these measures (see for instance Terracciano *et al.,* 2010).

Future work

There are several issues that deserve future research attention. First, our finding of a greater increase in serum BDNF concentrations in the course of antidepressant treatment is associated with a larger decrease in depression symptom severity may fuel work into the temporal dynamics between BDNF expression and treatment efficacy. It would be interesting if future studies could address early changes in the course of (non-)pharmacological treatment, a notion for which some evidence exists (Lang *et al.,* 2006; Machado-Vieira *et al.,* 2009; aan het Rot *et al.,* 2012). Besides, the prediction of how successful a given treatment will be, based on changes in serum BDNF concentrations (*i.e., a treatment biomarker*) is clinically relevant (see for instance Schmidt *et al.,* 2011). In our meta-analysis we did not have the possibility to address this because most of the included studies reported on pre- and post BDNF concentrations only. Another venue for future investigations regards the distinction between the pro- and the mature BDNF variant. The ELISA kits that currently are in use to quantify BDNF are not sensitive enough to make this distinction. Given the proposed opposing effects of these two BDNF variants (proBDNF is believed to induce apoptosis; Park and Poo, 2013) it would be interesting to study pro/mature BDNF ratios and whether these differ among diagnostic groups. The tools hereto were only recently developed and validated (Yoshida *et al.,* 2012).

With regard to future work on peripheral BDNF concentrations we finally wish to note that analyses would gain credibility if they were controlled for relevant confounding factors and performed using data (preferably within-subject) on a sufficiently large sample (*N* ~ 150, according to our power-analyses).

Concluding remarks

Our meta-analyses (aggregating 179 effect-size estimates; *N* = 9,484) initially yielded support for the claim that alternations in serum BDNF concentrations are peripheral manifestations of depression. This is not new. The important contribution of our work however is that we clearly show that between-study heterogeneity, underpowered designs, and publication bias are at play that together give rise to inflated

effect-size estimates. Together this suggest that the evidence base for the claim that altered serum BDNF concentrations are peripheral manifestations of depression is slimmer as was initially thought and amidst a lot of noise.

CHAPTER 6

Gender specific associations of serum BDNF concentrations in anxiety

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SIGNIFICANCE: Anxiety disorders mimic depression to a great extent, so it was expected that serum BDNF concentrations would be low in the patients with such an illness. In this well-powered study we could not confirm this expectation, exepct for somewhat lower BDNF concentrations in female patients. This gender specific finding may suggest that BDNF is involved in the pathophysiology of anxiety in women or, not unlikely, a female specific artifact of being anxious.

ABSTRACT

Whereas animal models indicate that Brain-Derived Neurotrophic Factor (BDNF) plays a role in anxiety-related behavior, little is known about BDNF in patients with an anxiety disorder. We therefore tested the hypothesis that serum BDNF concentrations are low in patients with an anxiety disorder as compared to healthy controls. We further examined the associations of gender and some of the clinical characteristics of anxiety with serum BDNF concentrations. Hereto, serum BDNF concentrations were determined in 393 unmedicated patients with social anxiety disorder, panic disorder, agoraphobia, and generalized anxiety disorder (66.7% females) and in 382 healthy controls (62.0% females). Overall, there were no differences in BDNF concentrations among patients and controls, regardless of type of anxiety disorder. Analyses stratified by gender, however, revealed that female patients had lower concentrations of BDNF relative to female controls ($P < 0.05$, effect size (Cohen's d) = 0.19), which was stronger in female patients with > 1 anxiety disorder ($P < 0.01$, $d = 0.32$). BDNF concentrations were similar among male patients and male controls and unrelated to the clinical characteristics of anxiety. Our results mirror preclinical findings indicating that gender plays a role in the association between BDNF and anxiety and suggest that BDNF might be involved in the pathophysiology of anxiety in women.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF), a neurotrophin that regulates neuronal survival and plasticity (Bramham and Messaoudi 2005), has been attracting growing attention in relation to major depressive disorder. The *neurotrophin hypothesis of depression* predicts that aberrant support by BDNF is associated with neuronal atrophy and an increased risk of depression (Duman *et al*., 1997; Duman and Monteggia 2006). Consistent with this neurobiological concept on depression are the replicated findings that BDNF concentrations are low in central and peripheral tissues during a depressive episode (Dwivedi *et al*., 2003; Molendijk *et al*., 2010). Depression is often accompanied by anxiety and it is believed that these disorders share similarities in their etiology and pathophysiology (Kendler *et al*., 1992, 1995; Klaassen *et al*., 1998; Maron *et al*., 2004; David *et al*., 2009). Therefore, some authors sought to extend the neurotrophin hypothesis of depression to the anxiety disorders. The first attempts to investigate the link between BDNF and anxiety used rodent models for anxiety-like behavior. Chen *et al*. (2006) for example genetically manipulated male mice so that the secretion of BDNF from neurons got depleted, which was associated with behavior that resembled human anxiety. Findings of Monteggia *et al*. (2007) on the other hand, seem to indicate that the conditional knockout of BDNF is associated with decreased anxiety-like behaviour in female mice, while having no effect on anxiogenic behavior in male mice. Finally, Govindarajan *et al*. (2006) reported that an enhanced expression of BDNF had a facilitatory effect on anxiety-like behavior in male mice. Thus, these data, although intriguing, remain inconclusive with regard to whether the neurotrophin hypothesis also applies to anxiety like-behavior. However, the data do support sex differences with regard to the association between BDNF and anxiety-like behavior. Data on BDNF protein concentrations in humans with an anxiety disorder is limited to two relatively small-scale studies in patients with panic disorder. The evidence from these studies is conflicting. The first of these studies found similar BDNF concentrations in 42 patients with panic disorder as compared to 31 controls (Kobayashi *et al*., 2005), whereas the second found lower BDNF concentrations in 12 patients with panic disorder as compared to 12 controls (Strohle *et al*., 2010). Data on the associations between BDNF and other anxiety disorders than panic disorder, such as social anxiety disorder or generalized anxiety disorder, are not available. Such data, however, are relevant because it could increase our understanding of the pathophysiology that may underlie anxiety (Martinowich *et al*., 2007). Here we addressed this important issue and determined serum BDNF concentrations in 393 unmedicated patients (66.7% females) with social anxiety disorder, panic disorder, agoraphobia, and generalized anxiety disorder, or a combination of these disorders and in 382 healthy controls (62.0% females). All patients were currently free of depression. We tested the hypothesis that serum BDNF concentrations are low in patients with an anxiety disorder as compared to controls in analyses that were controlled for a range of demographical and behavioral confounders. We further performed analyses on gender differences with regard to serum BDNF concentrations. Finally, in our patient sample, we tested whether and to what extent the type of anxiety disorder, the severity of anxiety, the chronicity of anxiety, the age at onset of anxiety, and having had MDD were related to serum BDNF concentrations.

METHODS

Study population

The data analyzed are from the Netherlands Study of Depression and Anxiety (NESDA; see Penninx *et al*., 2008 for an overview). Briefly, the NESDA is a prospective cohort study on 2,981 persons (66.4%female, aged 18 through 65) who were recruited in specialized mental health care, primary care, and in the general population. Included in NESDA were persons with a current or a remitted anxiety and/or mood disorder and persons without a lifetime diagnosis of an anxiety or mood disorder. Persons with a psychotic, bipolar,

obsessive–compulsive or severe addiction disorder were not eligible. Diagnoses of anxiety disorders (*i.e.,* Social Anxiety Disorder (SAD), Panic Disorder (PD), Generalized Anxiety Disorder (GAD), and Agoraphobia (Agr), and depressive disorders (*i.e.,* major and minor depressive disorder and dysthymia) were determined on the basis of responses to the Composite International Diagnostic Interview 2.1 (CIDI) life-time version (Wittchen *et al*., 1991) according to the criteria set forth in the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV; APA 1994). The CIDI is a commonly used diagnostic instrument and has a high reliability (Wacker *et al*., 2006) and validity (Wittchen *et al*., 1991). At baseline, participants also underwent a medical examination and provided blood samples. All subjects gave written informed consent for the study that was approved by the Ethical Committees of the participating institutes. The sample that was examined here consisted of NESDA participants who were diagnosed with PD, SAD, Agr, GAD, or a combination of these disorders within the last 6 months and of healthy controls. To allow a study on the association of BDNF with anxiety without the confounding effects of depression and psychotrophic medication use (associations that our group previously confirmed, see Molendijk *et al*., 2010) we selected patients who were currently free of depression and who were untreated with anxiolytics (ATC code N05B) and antidepressants (ATC codes N05B, N06A, and N06AX; WHO 2010) and St John's Wort. Healthy controls were eligible for inclusion if they were free of life-time anxiety and mood disorders, not at high risk for these disorders because of a documented family history of these illnesses, and if they scored low on Beck's Anxiety Inventory (BAI; < 10; Beck *et al*., 1988) and on the Inventory of Depressive Symptoms (IDS; < 14; Rush *et al*., 1996). We included a total of 393 patients and 382 lifetime healthy controls.

BDNF protein measurements

Serum samples were obtained before 10:00 h after an overnight fast and stored at –85 C˚. Serum BDNF protein concentrations were measured, in duplicate, using the Emax Immuno Assay system from Promega according to the manufacturer ' s protocol (Madison, WI, USA) by one technician who was blind to clinical diagnoses. Measurement procedures are described in detail elsewhere (Bus *et al*., 2011). In brief, serum samples were diluted 100 times, and the resulting absorbency was read in duplicate using a Bio-Rad (Hercules, CA, USA) Benchmark microplate reader at 450 nm. The intra- and inter-assay coefficients of variation were within 3 and 9% respectively.

Clinical characteristics

Four clinical characteristics were examined with regard to their association with serum BDNF concentrations. These included the severity of anxiety symptoms, the chronicity of anxiety, the age at onset of anxiety, and a history of MDD. Continuous scores based on BAI (Beck *et al*., 1988) were used as a measure for the severity of anxiety symptoms. BAI is a 21-item self-report measure that has good validity (Kabacoff *et al*., 1997) and test–retest reliability (Beck *et al*., 1988). The measure for the chronicity of anxiety was based on the life chart interview, a commonly used method to describe the course of psychopathology (Lyketos *et al*., 1994). The life chart refreshes memory by determining life events that occurred during the last 4 years and based on these “memory anchors” subsequently assesses the presence of anxiety symptoms during this interval. This yields a score in the range 0 – 48 months with avoidance behavior and/or panic attacks during the past 4 years. The CIDI interview (Wittchen *et al*., 1991) served as source of information on the age at onset of anxiety (*i.e.,* the age in years when the first episode of anxiety occurred) and on the presence of a depressive episode in the past (> 6 months ago).

Covariates

Previously we described that age, body mass index (BMI), smoking, alcohol intake, physical activity, time of the day of blood draw (in minutes from 06:00 h), and number of months of serum storage are associated with serum BDNF concentrations (Bus *et al*., 2010; Molendijk *et al*., 2010). Therefore, we took the possible confounding role of these variables into account by including them as covariates in all analyses. Data on age, BMI (weight/height^2), smoking (dichotomized as smoker versus non smoker), and alcohol use (coded as non-drinker, 0–1 units a day, 1–2 units a day, and > 2 units a day) were collected using standard methods (Penninx *et al*., 2008). Information on physical activity was gathered using the international physical activity questionnaire (Craig *et al.,* 2003) and expressed as number of met-minutes (*i.e.,* the ratio of the amount of energy expenditure during activity to the energy expenditure at rest). Together, this set of variables will be referred to as the set of *basic covariates*.

Statistical analysis

Demographical and clinical characteristics between patients and controls were compared using analyses of variance and Students *t*-tests for continuous data and χ^2 tests for categorical data.

Analysis of variance (ANOVA) with correction for the set of basic covariates was performed to assess differences in serum BDNF concentrations between all patients with an anxiety disorder and healthy controls. This analysis was repeated with diagnosis and gender as factors (2 times 2 ANOVA: any anxiety diagnosis versus controls and gender) to explore whether serum BDNF concentrations were comparable among female and male patients and controls and to test a possible interaction between diagnosis and gender. Next, a multiple linear regression analysis was performed to investigate whether serum BDNF concentrations differed between the subtypes of anxiety diagnoses (*i.e.,* SAD, PD, Agr, GAD, or > 1 anxiety disorders) and controls. In this analysis the set of basic covariates was entered in a first step of regression and dummy variables coding for the presence of each of the anxiety disorders were entered in a second step. The control group served as reference category.

In the patient sample, a regression analysis, corrected for the basic covariates, was performed to assess whether serum BDNF concentrations were associated to the severity-, the chronicity-, and the age at onset of anxiety and a history of a depressive episode. Computations were performed in SPSS 18.0 (Chicago, IL). A *P*-value of < 0.05 (two-tailed) was considered as the threshold for statistical significance. Effect sizes on between-group comparisons were presented as standardized Cohen's *d* (Cohen 1988). Standardized regression weights (β values) were used as an index of the strength and the direction of the associations that were obtained in the regression analyses. Tolerance of the predictors and normality of error variances was verified in all regression models.

RESULTS

Sociodemographic and clinical characteristics

Table 1 ↓ displays the demographical and clinical characteristics of patients and controls. Patients were on average younger, received fewer years of education, and were more likely to smoke and to use alcohol as compared to controls.

Serum BDNF concentrations in patients and controls

ANOVA (any anxiety diagnosis versus controls) revealed no main effect of diagnosis (mean BDNF anxiety = 9.31, *SD* = 3.38 versus healthy controls = 9.49, *SD* = 3.18, *P* = 0.49). This analysis was repeated with gender as additional factor (2 times 2 ANOVA: any anxiety diagnosis versus controls times gender) to explore whether serum BDNF concentrations were comparable among female and male patients and controls and to test an interaction between diagnosis and gender. Adding the factor gender to the analysis revealed a

diagnosis-gender interaction ($F_{1,754} = 4.02$, $P = 0.05$) apart from a main effect of gender ($F_{1,753} = 4.24$, $P = 0.05$), with males having higher serum BDNF concentrations than females. Pair-wise comparisons on least square differences revealed that female patients had lower concentrations of BDNF (mean = 8.90, *SD* = 3.24) relative to female controls (mean BDNF = 9.49, *SD* = 3.20; $t_{485} = 2.02$, $P = 0.05$, $d = 0.19$) and to male patients (mean BDNF = 9.94, *SD* = 3.44; $t_{376} = 3.16$, $P = 0.01$, $d = 0.30$). Male controls had BDNF concentrations (mean = 9.51, *SD* = 3.10) that were comparable to those of female controls ($P = 0.95$) and to male patients ($P = 0.28$). Importantly, possible confounds that might have had occurred because of between-group differences in age, educational attainment, smoking, and alcohol use were statistically controlled for. Mean corrected BDNF concentrations are plotted in **Figure 1 ↓** for persons with an anxiety disorder and healthy controls by gender.

Table 1. Demographic and clinical characteristics (mean ± standard deviation or percentages) by diagnosis and gender

		Patients (*n* = 393)		Controls (*n* = 382)		*P*-value
		Female (*n* = 262)	Male (*n* = 131)	Female (n = 237)	Male (*n* = 151)	
Age		40.1 ± 13.2	43.1 ± 12.9	44.1 ± 12.3	48.3 ± 11.9	< .001 [a, b, c, d]
Education (years)		12.0 ± 3.2	12.2 ± 3.3	13.3 ± 3.2	13.5 ± 3.5	< .001 [c, d]
Body Mass Index		24.7 ± 5.0	25.9 ± 4.4	24.8 ± 4.8	26.3 ± 4.1	.41 [a, b]
Smoker	%	39.8	36.5	14.1	20.1	< .001 [a, c, c]
Physical activity [1]		3.6 ± 2.9	3.8 ± 3.4	3.8 ± 2.9	3.8 ± 3.2	.94
Alcohol Use						
Non-drinker	%	51.3	31.9	60.5	48.2	< .01 [a, b, c, d]
Drinker 1-2 units a day	%	45.5	55.7	38.5	46.8	< .01 [a, b, c, d]
Drinker > 2 units a day	%	3.2	12.4	1.0	5.0	< .01 [a, b, d]
Social anxiety disorder	%	52.2	48.9	NA	NA	.47
Panic disorder [2]	%	46.2	38.9	NA	NA	.17
Generalized anxiety	%	17.6	22.1	NA	NA	.27
Agoraphobia	%	17.2	14.5	NA	NA	.34
> 1 anxiety disorder	%	17.6	22.1	NA	NA	.12
Anxiety characteristics						
Anxiety severity, BAI		15.2 ± 9.3	12.4 ± 8.8	2.7 ± 2.9	1.6 ± 2.2	< .001 [a, b, c, d]
Age at onset of anxiety		20.1 ± 12.6	21.9 ± 13.3	NA	NA	.15
Chronicity of anxiety [3]		22.6 ± 20.1	20.6 ± 20.2	NA	NA	.12
History of depression	%	45.0	32.1	NA	NA	< .01
BDNF (ng/ml)		8.9 ± 3.2	9.9 ± 3.4	9.5 ± 3.2	9.5 ± 3.1	< .05 [b, c]

Abbreviations: BAI; Beck's Anxiety Inventory, BDNF: Brain-Derived Neurotrophic Factor
[1] Mean met-minutes (*i.e.,* ratio of energy expenditure during activity to energy expenditure at rest)
[2] Percentages do not add up to 100% due to comorbidity among the anxiety disorders.
[3] Number of months with anxiety symptoms in the past 4 years
[4] Mean BDNF levels, corrected for the basic covariates (see Method section)
[a] Indicates a statistical significant difference ($P < .05$) between female and male controls
[b] Indicates a statistical significant difference ($P < .05$) between female and male patients
[c] Indicates a statistical significant difference ($P < .05$) between female controls and female patients
[d] Indicates a statistical significant difference ($P < .05$) between male controls and male patients

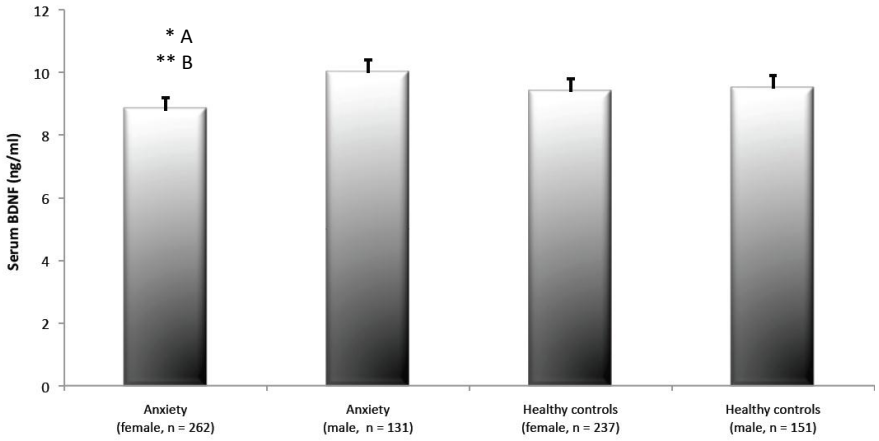

Figure 1. Plotted are mean serum BDNF concentrations by diagnosis and gender. Error bars reflect the *SEM*.
Serum BDNF concentrations are low in female patients with an anxiety disorder as compared with female controls (A: $d = 0.19$) and male patients with an anxiety disorder (B: $d = 0.30$).
The diagnosis-gender interaction effect is significant at $P < .05$.

* Denotes statistical significance at $P < 0.05$.

The role of gender (indicated by the main effect of gender and the diagnosis–gender interaction) was ascertained by modeling all subsequent analyses separately for female and male subjects as well as for the whole sample. Using multivariable regression analyses, with correction for the basic covariates, we investigated whether BDNF concentrations differed between the subtypes of anxiety diagnoses (*i.e.,* SAD, PD, Agr, GAD, or > 1 anxiety disorder) versus healthy controls. Tolerance of the predictors was close to 1 (range: 0.91 – 0.97), indicating no redundancy among the predictors. Furthermore, error variances were normally distributed. Overall, there were no differences in serum BDNF concentrations between each of the types of anxiety diagnoses versus controls (see **Table 2 ↓**). In analyses stratified for gender it appeared that BDNF concentrations were low in female patients with > 1 anxiety diagnosis as compared to female controls ($P < 0.05$). BDNF concentrations of male patients with > 1 anxiety diagnosis, instead, were somewhat higher as compared to male controls ($P = 0.10$). The differences among female patients and female controls and among female and male patients in serum BDNF concentrations, as they were found in analysis of covariance, thus were largely driven by patients who had > 1 anxiety diagnosis. Indeed, effect sizes for the comparison of corrected BDNF concentrations among female patients versus female controls ($d = 0.19$) became larger when comparing BDNF concentrations of female patients with > 1 anxiety diagnosis versus female controls ($d = 0.32$). Similarly, the difference among female and male patients ($d = 0.30$) also became more apparent when comparing BDNF concentrations of female patients with > 1 anxiety diagnosis versus male patients with > 1 anxiety diagnosis ($d = 0.66$).

Table 2. Results of univariable correlation and multivariable regression analyses on serum BDNF levels in patients with an anxiety disorder contrasted versus healthy controls ($n = 775$)

	Whole sample ($n = 393$)			Female ($n = 262$)			Male ($n = 131$)		
	r [1]	*B* ± *SEM*	ß	r [1]	*B* ± *SEM*	ß	r [1]	*B* ± *SEM*	ß
Social phobia	-.04	-0.28 ± 0.27	-0.04	-.08*	0.78 ± 0.54	0.10	.03	0.11 ± 0.48	0.02
Panic disorder	.02	0.14 ± 0.30	-0.02	.03	0.50 ± 0.53	0.07	.12*	0.77 ± 0.52	0.09
Agoraphobia	.01	-0.16 ± 0.44	0.01	-.02	0.62 ± 0.72	0.06	.06	0.64 ± 0.79	0.05
Generalized anxiety	-.01	-0.01 ± 0.40	-0.01	-.03	0.19 ± 0.81	0.02	.04	0.40 ± 0.64	0.04

[1] Spearman's *rho* with variables coded as: 1 = not present, 2 = present.
* Statistical significance at $P < .05$
Note. The healthy controls served as the reference category in the multivariable model. *B*'s and ß's thus represent the association of the specific anxiety disorders versus having no anxiety disorder

Additional regression analyses, corrected for the set of basic covariates were run to elucidate whether variation in some of the clinical characteristics of anxiety or a history of depression could account for variation in serum BDNF concentrations. The clinical characteristics correlated among each other (ranging from –0.04 to 0.28), yet the tolerance of the individual predictors was close to 1 (range: 0.80 to 0.98) indicating no redundancy among the predictors. Furthermore, error variances were normally distributed. The regression showed no association of the severity and chronicity of anxiety, and having had an episode of major depression with serum BDNF concentrations (see **Table 3 ↓**). Age at onset of anxiety was positively associated with serum BDNF in univariable analyses, which seemed to be largely driven by the male patients in the sample (see **Table 3 ↓**). However, also in male patients this association did not reach full statistical significance and in multivariable analyses only a trend-like finding (P = 0.09) was observed. Finally, the difference between female patients and male patients in serum BDNF concentrations was observed in all regression models. Hence the gender difference in serum BDNF concentrations could not be attributed to between-gender differences in the demographical, behavioral, and clinical variables on which we focused in the current study.

Table 3. Results of univariable correlation and multivariable regression analyses on the association of the clinical facets of anxiety with serum BDNF levels in patients with an anxiety disorder (n = 393)

	Whole sample (n = 393)			Female (n = 262)			Male (n = 131)		
	r [1]	$B \pm SEM$	ß	r [1]	$B \pm SEM$	ß	r [1]	$B \pm SEM$	ß
Anxiety characteristics [1]									
Anxiety severity	-.01	-0.01 ± 0.02	-0.01	-.01	-0.01 ± 0.02	-0.02	.07	0.01 ± 0.04	0.02
Age at onset	.11*	0.02 ± 0.02	0.06	.06	-0.01 ± 0.02	-0.02	.16*	0.06 ± 0.03	0.22*
Chronicity of anxiety	.07	0.02 ± 0.01	0.09	.06	0.01 ± 0.01	0.08	.09	0.01 ± 0.02	0.06
>1 anxiety disorder [2]	-.07	-0.02 ± 0.41	-0.05	-.12*	-0.84 ± 0.01	-0.12†	.11	0.98 ± 0.81	0.12
History of depression [2]	-.03	-0.02 ± 0.35	-0.02	.01	0.01 ± 0.08	0.01	-.07	-0.05 ± 0.15	-0.03

[1] Pearson's r when continuous variables are involved and Spearman's *rho* if dichotomous are involved
[2] Dichotomous variables are coded as: 1 = not present, 2 = present
† Indicates a trend at P = .07. * Statistical significance at P < .05

DISCUSSION

The primary goal of this study was to test the hypothesis that serum BDNF concentrations are low in patients with an anxiety disorder as compared to healthy controls. Our results, controlled for a range of demographical and behavioral variables, did not confirm this hypothesis as overall no differences between patients with an anxiety disorder (*i.e.,* social anxiety disorder, panic disorder, agoraphobia, and generalized anxiety disorder) and healthy controls were found in the amount of BDNF in blood serum. Given these data, it seems unlikely that BDNF is involved in the pathophysiology of anxiety disorders *per se*. Nevertheless, additional analyses on gender differences in serum BDNF concentrations revealed that female patients with an anxiety disorder had low serum BDNF concentrations as compared to female controls and to male patients. BDNF concentrations in male patients tended to be slightly higher as compared to male controls. BDNF concentrations among female and male controls were similar. Thus, our gender specific finding, showing lower concentrations of BDNF only in female and not in male patients with an anxiety disorder, might point in the direction that BDNF is related to the pathophysiology of anxiety in female but not in male patients. Other than the here reported data, very little is known on the relation between BDNF and human anxiety. As referred to in the introduction, to date, only two studies addressed this issue and these studies present conflicting results. Kobayashi *et al*. (2005) found no differences in serum BDNF concentrations between patients with panic disorder and healthy control subjects. Our analyses that were run in the whole sample confirmed this finding. Strohle *et al*. (2010) on the other hand did find lower

concentrations of BDNF in patients with panic disorder as compared to healthy control subjects. The sample that was studied by Strohle *et al*. however consisted mostly of females (75%). Therefore it could be that the large proportion of females drove the results that were reported in this particular paper.

Interestingly, our findings are in agreement with the observation that anxiety in female mice is more susceptible to changes in BDNF than in males (Monteggia *et al*., 2007). Of note is that the methods that were used to manipulate BDNF expression in these preclinical studies were rigorous (*e.g.,* a complete knockout or ~ 10-fold over-expression of BDNF; Govindarajan *et al*., 2006; Monteggia *et al*., 2007). Therefore, the outcomes of these studies might lack the necessary ecological validity to be directly comparable to the outcomes of studies using human subjects (Groves 2008). However, our gender-specific findings also compare well with some studies in patients with depressive disorders showing lower concentrations of BDNF in female depressed patients as compared to male depressed patients (Karege *et al*., 2002a; Huang *et al*., 2008). The origins of our gender specific findings are unknown. Here, and also in a previous study on depressed subjects (Molendijk *et al*., 2010) we found that the differences in serum BDNF concentrations between female and male patients were not driven by demographical (*e.g.,* age), behavioral (*e.g.,* smoking), or clinical (*e.g.,* severity) variables. In the current study, we further found that the difference between female and male patients could not be attributed to a specific subtype of anxiety. A general deduction from this, and from our finding that serum BDNF concentrations are similar among female and male controls, is that the origins of our gender-specific findings may lie in a female specific associate of anxiety. One interesting candidate that might serve as an explanation for our gender specific findings is an explanation in terms of alternations in the expression of the ovarian hormone estrogen in females during the state of anxiety. The expression of estrogen typically is low in females with an anxiety disorder (Seeman, 1997; Almeida *et al*., 2005; Walf and Frye, 2006) and this might be of relevance here since estrogen is a signaling molecule upstream of BDNF that triggers the expression of BDNF (Scharfman and MacLusky, 2004; Begliuomini *et al*., 2007). Furthermore, estrogen has been shown to have therapeutic effects in psychiatric conditions such as major depression and schizophrenia (see for example Kulkarni *et al*., 2008; Young and Korszun, 2010), disorders in which peripheral BDNF concentrations also are low, as confirmed by recent meta-analyses (Sen *et al*., 2008; Green *et al*., 2010). Therefore, the interaction of estrogen with BDNF might be of importance in our understanding of low BDNF concentrations in female patients in general.

In addition to gender-specific findings we found that serum BDNF concentrations are similar across the subtypes of anxiety disorders and thus peripheral BDNF measurements do not have the specificity to categorize anxiety disorders. Furthermore, it should be noted that peripheral BDNF measurements lack specificity to categorize psychiatric disorders outside the spectrum of anxiety (see also Gass and Hellweg, 2010 for a review) as low concentrations of BDNF have been shown in depression (Karege *et al*., 2002a), schizophrenia (Green *et al*., 2010), and eating disorders (Nakazato *et al*., 2003). Interestingly, the number of anxiety disorder, on the other hand, did show associations with serum BDNF concentrations. In female patients serum BDNF concentrations tended to decrease as the number of anxiety disorders increased, whereas in male patients serum BDNF concentrations tended to increase as the number of anxiety disorders increased. Interestingly, some studies using predominantly female patients have shown a worse clinical course and a greater impairment in patients who suffer from multiple anxiety disorders (Bruce *et al*., 2005; Kroenke *et al*., 2007) and thus having multiple anxiety disorders might be considered an indication of anxiety severity. However, our findings that a higher symptom severity of anxiety or a more chronic course do not go along with lower concentrations of BDNF seems to suggest that no associations exist between BDNF and the severity of anxiety. In addition, a later age at anxiety onset appeared to be associated with higher concentrations of BDNF, particularly in male patients. However, in multivariate

analyses this association did not reach full statistical significance. Finally, we found a history of depression to be unrelated to serum BDNF concentrations in patients with a current anxiety disorder, which is in line with our previous finding that BDNF concentrations are low during a depressive episode but return to normal in the course of depression remission (Molendijk *et al*., 2010).

A salient strength of our study is that we report on a large sample of various anxiety disorders that allowed for analyses stratified by gender. Moreover, all analyses were controlled for possible confounding effects of various demographical and behavioral variables, showing that the current findings could not be explained by such factors. Moreover, we could eliminate the confounding effects of depression and the use of psychotrophic medication. Thus, we believe that our results advance the understanding of the role of BDNF in anxiety. Notwithstanding this, we do wish to emphasize some limitations of our study. First, we evaluated correlative associations and therefore we do not know whether our main finding of low concentrations of BDNF in female patients with an anxiety disorder are causally involved in anxiety or whether they are merely a consequence of being anxious. Furthermore, although some between-group differences in the current study reached statistical significance, the effect sizes on these associations typically were small leading to the question whether or not our findings are of any clinical relevance. Yet another limitation might be that we studied easily accessible serum BDNF concentrations and can only assume that these measurements mirror the amount of BDNF in the brain (Sartorius *et al*., 2009; Klein *et al*., 2010). This, however, only is an assumption since there are many possible other sources of BDNF in blood serum (Karege *et al*., 2002b). Given that especially platelets constitute a source of peripheral BDNF concentrations, it might be worthwhile to control for platelet count in future studies on between-group differences in serum BDNF concentrations (Karege *et al*., 2005; Ziegenhorn *et al*., 2007). Finally, we studied BDNF concentrations in isolation of other hormones, neurotransmitters, and receptors that might interact with BDNF and as such could have explained the associations that we observed (Kapczinski *et al*., 2010).

In sum, this large-scale study in patients with anxiety disorders shows that serum BDNF concentrations are low in female patients with an anxiety disorder but not in male patients with an anxiety disorder. These results were not driven by differences in demographical, behavioral, or clinical variables and thus suggest that low concentrations of BDNF might be specifically related to the pathophysiology of anxiety in females. Future research is needed to clarify whether these lower concentrations of BDNF in females contribute to anxiety or whether they are merely a consequence of having one or more anxiety disorders. Furthermore, the clinical significance of our findings requires examination and hereto longitudinal studies are needed.

CHAPTER 7

The impact of childhood abuse and recent stress on serum BDNF and the moderating role of val^{66}met

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SIGNIFICANCE: The axiom that prevails in explaining depression related alternations in BDNF expression is trauma/stress exposure. We do not find evidence for this except for a negative correlation between recent stress exposure and serum BDNF concentrations, explaining only ~ 1 percent of the variance. We do find a val^{66}met - trauma interaction effect on serum BDNF concentrations, which in contrast to expectations had no effect on behavior. The extent to which this interaction may be important (on various levels of functioning) remains be elucidated.

ABSTRACT

Recent findings show lowered Brain-Derived Neurotrophic Factor (BDNF) concentrations in major depressive disorder. Exposure to stressful life events may (partly) underlie these reductions in BDNF, but little is known about the effects of early or recent life stress on BDNF concentrations. Moreover, the effects of stressful events on BDNF concentrations may in part be conditional upon a common variant on the BDNF gene (val^{66}met; rs6265), with the met allele being associated with a decrease in activity dependent secretion of BDNF compared to the val allele. We investigated in 1,435 adults with lifetime MDD the impact of childhood abuse and recent life events on serum BDNF concentrations and assessed whether the impact of these events was moderated by the BDNF val^{66}met polymorphism. Overall, BDNF met carriers had reduced serum BDNF concentrations when exposed to childhood abuse in a dose-dependent way. Exposure to recent life events was also associated with decreases in BDNF concentrations, but this was independent of BDNF val^{66}met. Moreover, when not exposed to childhood abuse, met carriers had higher BDNF concentrations than the val/val individuals, who did not show decreases in BDNF associated with childhood abuse. Finally, these findings were only apparent in the depressed group without comorbid anxiety. These gene–environment interactions on serum BDNF concentrations suggest that met carriers are particularly sensitive to early stressful life events, which extends previous findings on the moderating role of the BDNF val^{66}met polymorphism in the face of stressful life events.

INTRODUCTION

Major depression is one of the most common psychiatric disorders, which involves dysregulation of affect, motivation, appetite, sleep, and cognitive dysfunctions, resulting in impairments in several aspects of life. An accumulating body of research indicates that depression is often the result of the interplay between genetic vulnerability and environmental factors (Kendler, 2005). In particular, childhood abuse is a significant etiological factor in the development and persistence of depression across the life cycle (Charney, 2004; Heim and Nemeroff, 2001; McLaughlin *et al*., 2010; Spinhoven *et al*., 2010). Moreover, exposure to stress during adulthood (for example, divorce or job loss) often precipitates or exacerbates depressive symptoms (Kendler *et al*., 1999). In some instances, stressful events in adulthood interact with stressful events that occurred early in life to contribute to the process of stress sensitization (Post 2007).

Given its major contribution to the burden of disease, it is important to identify underlying biological mechanisms that might lead from stress exposure to depression. One of the neurobiological changes that may be triggered by both chronic and acute stress is a down-regulation of Brain-Derived Neurotrophic Factor (BDNF). The neurotrophin BDNF is a critical regulator of the formation, plasticity, and integrity of neurons in brain circuits that regulate emotion (Angelucci *et al*., 2005). In animals, exposure to stress early in life (*e.g.,* maternal separation) has been found to induce a relative decrease in the expression of BDNF and to subsequent neuronal atrophy and degeneration in the hippocampus and the cortex, which can persist into adulthood (Smith *et al*., 1995; Roceri *et al*., 2004; Song *et al*., 2006). According to the *neurotrophic hypothesis of depression*, reductions in BDNF expression may account for the pathophysiology of depression (Duman and Monteggia, 2006). Consistent with this idea, several studies found decreased central (Karege et al., 2005) and peripheral concentrations of BDNF (Molendijk *et al*., 2011) in depressed patients. More specifically, in line with this, one study found lowered plasma BDNF in depressed women with a history of childhood neglect compared to non-abused depressed women and controls (Grassi-Oliveira *et al*., 2008). A study among bipolar patients reported similar reductions in serum BDNF concentrations in patients exposed to stressful life events (Kauer-Sant'Anna *et al*., 2007).

A common Single-Nucleotide Polymorphism (SNP) on the BDNF gene is val^{66}met. Val^{66}met refers to a valine (val) to methionine (met) insertion at codon 66 (Egan *et al*., 2003). This SNP affects intracellular processing and secretion of BDNF, with the met allele being associated with a decrease in activity-dependent secretion of BDNF compared to the val allele (Egan *et al*., 2003). Most studies have compared carriers of a met allele (val/met) with individuals who are homozygous for the val allele (val/val) because individuals who are homozygous for the met allele (met/met) are rare in Caucasians (~ 4%). In general, these studies have shown that met carriers have lower hippocampal gray matter (Pezawas *et al*., 2004; Bueller *et al*., 2006) and poorer episodic memory performance (Egan *et al*., 2003) compared to individuals homozygous for the val allele. Moreover, several studies have reported that met carriers are more vulnerable to the effects of childhood abuse compared to individuals who are homozygous for the val allele in terms of depressive symptoms (Kaufman *et al*., 2006; Wichers *et al*., 2008) and hippocampal gray matter (Gatt *et al*., 2009). Whereas variations on the BDNF gene seem to play an important role in depression, little is known on how variations on the BDNF polymorphism val^{66}met may influence serum BDNF concentrations of individuals who have been exposed to childhood abuse and/or recent negative life events. A closer examination of the impact of childhood abuse and recent life stress and the moderating role of variations on the BDNF polymorphism val^{66}met on blood-derived BDNF concentrations may help to elucidate the neurobiological changes that underlie the susceptibility of developing depression after exposure to stressful life events. Therefore we investigated, cross-sectionally, the impact of childhood abuse and recent life events on serum BDNF concentrations in a large sample of individuals with lifetime depression and assessed whether the impact of these stressful life events was moderated by variations on

the BDNF val^{66}met polymorphism. Based on findings in animals and humans, we hypothesized that childhood abuse and recent life events would reduce serum BDNF concentrations, particularly in met carriers of the BDNF val^{66}met polymorphism.

METHOD

Patients and sample collection

Participants were derived from the NESDA (for details on the design, objectives, and protocol see Penninx *et al*., 2008). In brief, NESDA is a prospective cohort study (*N* = 2,981) that recruited subjects with a current depression and/or an anxiety disorder, patients with depression and/or an anxiety disorder in remission, and healthy controls without a history or current depression or anxiety disorder in specialized mental health care, primary care, and the general population. A general inclusion criterion was an age of 18 through 65 years. Excluded were individuals with a primary diagnosis of psychotic, obsessive compulsive, bipolar, or severe addiction disorder (requiring care in specialized addiction clinics). A second exclusion criterion was not being fluent in Dutch. At baseline, participants provided blood and underwent a medical examination. The study protocol was approved by the Ethical Review Board of the VU University Medical Center and by local review boards of each participating institute. After full information about the study was provided, written informed consent was obtained from all participants. From the NESDA baseline sample, we selected 1,435 participants (48.1%), with a mean age of 42.2 years (± 12.4) and 30.7% (*n* = 440) males. To investigate individuals with a vulnerability to depression, our selection was based on the following criteria: (1) participants had to have a current or lifetime diagnosis of depression; (2) genomic data, data on serum BDNF concentrations, and measurements of childhood abuse and recent stress had to be available; and (3) participants had to be of North-European descent. DSM-IV diagnoses (APA, 1994) of major depression and anxiety disorders (generalized anxiety, social phobia, panic with or without agoraphobia, or agoraphobia) were determined by means of the Composite Interview Diagnostic Instrument (CIDI; Wittchen *et al*., 1991) that was administered by trained research staff. The CIDI has high reliability (Wacker *et al*., 2006) and validity (Wittchen *et al*., 1991). Depression symptom severity was assessed using the Inventory of Depressive Symptoms Self-Report version (IDS; Rush *et al*., 1996). The use of antidepressants was gauged on by self-report and drug container observation.

Childhood abuse was assessed retrospectively using a semi-structured childhood trauma interview, previously used in the Netherlands Mental Health Survey and Incidence Study (de Graaf *et al*., 2004a, b). In this interview, participants were asked whether they had experienced before the age of 16 years one of the following types of trauma: emotional neglect, psychological, and physical and/or sexual abuse. After an affirmative answer, details on the frequency of these events and the perpetrators involved were asked for. Because of the large overlap between emotional neglect and emotional abuse, the two types of abuse were merged together as *emotional abuse*. Answers were coded as zero, one, two, or three reported types of childhood abuse. The mean number (± Standard Deviation [*STD*]) of childhood abuse types was 1.12 ± 1.15, with 42.7% (*n* = 613) reporting no childhood abuse, 21.6% (*n* = 310) reporting one type of childhood abuse, 17.1% (*n* = 246) reporting two types of childhood abuse, and 18.5% (*n* = 266) reporting three types of childhood abuse. For the main analysis of variance (ANOVA), the presence of childhood abuse was defined as 0 versus ≥ 1 type of CA. For dose–response analyses, individuals were divided into three categories: individuals reporting no childhood abuse, one type of childhood abuse, and two or more types of childhood abuse.

The occurrence of 12 recent stressful life events (**'***recent stress***'**) was assessed using the List of Threatening Events Questionnaire (LTE-Q; Brugha *et al*., 1985; Brugha and Cragg, 1990). These events reflect the presence of life stressors during the past year, such as serious illness and injury, death of close

friend or relative, unemployment, major financial loss, and loss of important relationships. The LTE-Q has good test–retest reliability, high agreement between participant and informant ratings, and good agreement with interview-based ratings (Brugha and Cragg, 1990). Answers were coded as the total number of life events. The mean number (± *STD*) of reported stressful life events was 0.68 (± 1.0), with 58.4% (*n* = 838) reporting no life events, 23.6% (*n* = 338) reporting one event, 12.1% (*n* = 173) reporting two events, 4.5% (*n* = 64) reporting three events, 0.6% (*n* = 9) reporting four events, 0.6% (*n* = 9) reporting five events, 0.2% (*n* = 3) reporting six events, and 0.1% (*n* = 1) reporting seven events. For the main analyses, *recent stress* was defined as 0 versus ≥ 1 incident(s) of (a) stressful life events during the preceding year, whereas for the dose–response analyses, individuals were divided into three groups: individuals reporting no life event, one life event, and ≥ 2 life events in the past year.

Genotyping

For detailed descriptions on the procedures according to which genotyping was performed, we refer to Boomsma *et al*., (2008). The val^{66}met polymorphism (Dibisnp RS6265) was extracted from whole genome data using PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink). Val^{66}met was in the equilibrium as stated by Hardy and Weinberg (*P* = .28). Moreover, genotype frequencies (val^{66}val 65.5%, val^{66}met 32.5%, and met^{66}met 2%) were similar to those reported in previous studies on Caucasian populations (Gatt *et al*., 2009; Lang *et al*., 2009). Individuals who were homozygous for the met allele were merged with the heterozygous individuals into a group of met carriers (*n* = 495) and compared to homozygous val carriers (*n* = 940).

BDNF measurements

Fifty milliliters of blood was withdrawn into vacuum tubes between 07:30 a.m. and 09:30 a.m. after an overnight fast. Following blood collection, serum was separated and stored at −85° C until it was assayed. BDNF protein concentrations were measured using the Emax ImmunoAssay system from Promega according to the manufacturer's protocol (Madison, WI, USA). Greiner Bio-One high-affinity 96-well plates were used. Serum samples were diluted 100 times, and the absorbency was read in duplicate using a Bio-Rad Benchmark microplate reader at 450 nm. Serum BDNF protein concentrations were expressed in nanograms (ng) per milliliter. The intra-assay and inter-assay coefficients of variation were within 3% and 9%, respectively. Prior to analyses, BDNF values that were three SD above the mean (*n* = 5, 0.35%) were trimmed to a value of the mean plus three *SD's*. One BDNF value (0.07%) was below the reliable detection limit of the ELISA kit of 1.56 ng/ml and was set at the lower detection limit of 1.56 ng/ml.

Statistical analysis

Ancova's and χ^2 tests were used to determine between-group differences in demographical and clinical features. Estimates of the main and interaction effects of childhood abuse, recent stress, and val^{66}met on serum concentrations of BDNF were performed using 2 (childhood abuse: yes/no) times 2 (recent stress: yes/no) times 2 (val^{66}met: val/val versus met carriers) ancova. Childhood abuse and recent stress were entered as dichotomous variables in order to have a maximal number of subjects in each cell. Since gender, age, years of education, symptom severity of depression, presence of current depression versus remitted depression, presence of a current co-morbid anxiety disorder, use of an antidepressant, exact time of morning blood withdrawal, and the duration of serum storage have been discussed as potential sources of between-subject variation in BDNF concentrations (Trajkovska *et al*., 2007; Begliuomini *et al*., 2008), we statistically controlled for their possible confounding effects by adding these variables as covariates to the analysis. Significant interactions were followed up by independent *t*-tests.

Secondly, because recent studies suggest that the symptomatology and causal pathways for depression without co-morbid anxiety disorder may be quite distinct to those for depression with co-morbid anxiety disorder(s)(see Gatt *et al*., 2009), we repeated the same 2 (childhood abuse: yes/no) times 2 (recent stress: yes/no) times 2 (val^{66}met: val/val versus met carriers) ancova in participants with (lifetime) depression without co-morbid anxiety (depression − anxiety, *n* = 401) and individuals with (lifetime) depression and co-morbid anxiety disorders (depression + anxiety, *n* = 1,033), separately. Finally, to asses dose–response relationships between childhood abuse and recent stress and BDNF concentrations, additional Ancovas were conducted in the case of significant main effects of childhood abuse and/or recent stress or interactions with val^{66}met, based on three categories (no childhood abuse or recent life events versus one type of childhood abuse or recent life event versus two or more types of childhood abuse or recent life events). Computations were performed in PASW version 18.0 (PASW, Chicago, IL, USA). Statistical significance was set at $P < .05$ (two-sided). Effect sizes were presented as Cohen's *d* (Cohen 1988).

RESULTS

Demographics

Table 1 ↓ shows the demographical and clinical characteristics by val^{66}met, reported history of CA, and recent stressful life events. Exposure to childhood abuse and recent stressful life events was independent of BDNF genotype ($P = .13$ and $P = .74$, respectively). Exposure to recent stressful life events tended to be reported somewhat more often in individuals with a history of childhood abuse ($P = .07$). Individuals who were homozygous for the val allele had more years of education compared to individuals who carried a met allele. Individuals who reported childhood abuse were of older age and more likely to be female, to have a current episode of depression, to have more chronic depression, to have greater symptom severity of depression, and co-morbid anxiety and alcohol use disorders compared to individuals who did not report childhood abuse. Individuals reporting recent life events were of younger age, had less years of education, and were more likely to smoke, to have a current episode of depression, and to have chronic depression, greater symptom severity of depression, and a co-morbid anxiety disorder (see **Table 1 ↓**). No other main effects or val^{66}met times childhood abuse, val^{66}met times recent stress, val^{66}met times childhood abuse times recent stress interactions were found.

Table 1. Demographic and clinical characteristics (mean ± *STD* or percentage) by val^{66}met and exposure to childhood abuse and recent stressful events (*N* = 1,435)

	val^{66}val (*n* = 940)				val^{66}met (*n* = 495)				
	No abuse (*n* = 388)		Abuse (*n* = 552)		No abuse *(n =225)*		Abuse *(n =270)*		
	No recent stress (*n* = 237)	Recent stress (*n* = 151)	No recent stress (*n* = 309)	Recent stress (*n* = 243)	No recent stress (*n* = 138)	Recent stress (*n* = 87)	No recent stress (*n* = 154)	Recent stress (*n* = 116)	*P*-value
Male	38.0	32.5	25.9	26.3	31.2	37.9	31.8	27.6	< .05 [B]
Age (years)	42.3 ± 12.8	38.5 ± 13.7	44.1 ± 11.4	42.2 ± 11.8	41.8 ± 13.3	39.5 ± 12.7	44.4 ± 11.7	41.7 ± 12.5	< .01 [B,C]
Education (years)	12.3 ± 3.1	12.1 ± 3.2	12.2 ± 3.4	11.8 ± 3.3	12.1 ± 3.0	11.2 ± 3.1	12.0 ± 3.1	11.4 ± 3.3	.06 [A,C]
Body Mass Index	26.2 ± 4.9	25.1 ± 4.4	25.7 ± 5.3	26.0 ± 5.3	25.3 ± 4.9	25.4 ± 4.8	26.5 ± 5.4	25.7 ± 5.6	.23
Smoker	31.3	42.9	38.0	41.8	34.1	48.8	3.3	48.2	< .01 [C]
Alcohol dependent	13.9	13.6	22.0	23.9	18.8	14.9	20.8	29.3	< .01 [B]
Emotional abuse	NA	NA	93.8	94.8	NA	NA	94.4	96.3	.79
Physical abuse	NA	NA	27.8	34.9	NA	NA	27.1	40.4	< .05
Sexual abuse	NA	NA	38.2	29.3	NA	NA	29.2	38.5	.09
> 1 event of abuse	NA	NA	63.1	64.2	NA	NA	53.9	67.2	.10
> 1 event of recent stress	NA	47.7	NA	43.2	NA	58.6	NA	32.8	< .05 [B]
Current depression	46.4	54.3	57.9	68.3	59.4	54.0	54.5	69.8	< .01 [B,C]
Chronic MDD [1]	24.3	22.7	28.1	33.2	21.5	28.2	24.6	37.4	< .05 [B,C]
Depression severity	22.1 ± 12.8	24.3 ± 12.4	27.7 ± 13.2	31.2 ± 12.4	24.0 ± 13.5	23.2 ± 13.5	26.7 ± 13.1	29.7 ± 11.6	< .01 [B,C]
Comorbid anxiety [2]	30.0	28.5	41.4	49.8	33.3	33.2	35.1	46.6	< .01 [B,C]
Antidepressant use [3]	35.0	33.1	39.3	42.8	30.4	26.4	39.6	44.0	< .05 [B]

Abbreviations: BMI: Body Mass Index

[1] Included a diagnosis of social phobia, panic disorder with and without agoraphobia, agoraphobia, or generalized anxiety disorder. Comorbid anxiety was assessed using the CIDI interview

[2] Included the use of noradrenergic and specific serotonergic antidepressants, serotonin and norepinephrine reuptake inhibitors, selective serotonin reuptake inhibitors, St John's wort, and tricyclic antidepressants

[A] There is a statistically significant difference between the val^{66}val group and val^{66}met group

[B] There is a statistically significant difference between the no abuse group and the abuse group

[C] There is a statistically significant difference at between the no recent stress group and the recent stress group

Impact of childhood abuse, recent stress, and val^{66}met on serum BDNF concentrations

Using a 2 (childhood abuse: yes/no) times 2 (recent stress: yes/no) times 2 (val^{66}met: val/val versus met carriers) Ancova on serum BDNF concentrations, we found no main effect of childhood abuse (*P* = .38) nor a main effect of val^{66}met on BDNF concentrations (*P* = .33), but BDNF val^{66}met moderated the effects of childhood abuse on serum BDNF concentrations ($F_{1,\ 1,416}$ = 5.57, *P* = .01, see **Figure 1 ↓**). Met carriers reporting childhood abuse had significantly lower concentrations of BDNF compared to met carriers that did not report childhood abuse ($F_{1,\ 506}$ = 4.19, *P* = .04, *d* = 0.19), whereas individuals reporting childhood abuse who were homozygous for the val allele had similar concentrations of BDNF compared to homozygous val carriers without childhood abuse (*P* = .12). Furthermore, in individuals reporting no childhood abuse, met carriers had higher concentrations of BDNF compared to homozygous val carriers ($F_{1,\ 629}$ =3.88, *P* = .04, *d* = 0.19), while in the childhood abuse group, met carriers had similar concentrations of BDNF compared to homozygous val carriers (*P* = .22). Exposure to recent stressful life events did not affect BDNF concentrations (*P* = .79). No other interaction effects were found (all *P*-values > .10).

Dose–response associations between childhood abuse and BDNF

To investigate a dose–response association between CA and BDNF concentrations, an additional 3 (categories of childhood abuse: no childhood abuse [*n* = 613], one type of childhood abuse [*n* = 310], or two or more types of childhood abuse [*n* = 512]) times 2 (val^{66}met: homozygous val/val versus met carriers) ANOVA was conducted. An interaction was found between CA categories and val^{66}met ($F_{2,\ 1,418}$ = 2.99, *P* = .05). *Post-hoc* comparisons showed that BDNF concentrations were only low in met carriers when participants reported two or more types of childhood abuse compared to no childhood abuse (*P* = .032), but not when they reported one type of childhood abuse (*P* = .51), while in homozygous val carriers, no

main effect of childhood abuse categories was found (P = .29, data not shown).

Depression without comorbid anxiety

We repeated the 2 (C childhood abuse: yes/no) times 2 (recent stress: yes/no) times 2 (BDNF val^{66}met: val/val versus met carriers) Ancova on serum BDNF concentrations in individuals with an (lifetime) depression without a co-morbid anxiety disorder (n = 402). In this group, recent stress did affect BDNF concentrations ($F_{1, 383}$ = 7.19, P = .008, d = 0.29), indicating lower BDNF concentrations in individuals reporting one or more recent stressful life events (8.24 ± 3.20) compared to those who did not report negative life events (9.16 ± 3.06). Moreover, the interaction between childhood abuse and val^{66}met was also present in the depression - anxiety group ($F_{1, 383}$ = 9.77, P = .002), with met carriers who reported childhood abuse having significantly lower serum concentrations of BDNF compared to met carriers who did not report childhood abuse ($F_{1, 138}$ = 10.03, P = .002, d = 0.44), whereas individuals reporting childhood abuse who were homozygous for the val allele even had somewhat higher concentrations of BDNF compared to homozygous val carriers without childhood abuse ($F_{1, 258}$ = 3.82, P = .05, d = 0.23). Moreover, met carriers with a reported history of childhood abuse showed lower BDNF concentrations compared to homozygous val carriers with reported childhood abuse ($F_{1, 173}$ = 7.45, P = .007, d = 0.45), while met carriers reporting no childhood abuse had higher BDNF concentrations compared to the non-abused homozygous val carriers ($F_{1, 223}$ = 5.34, P = .02, d = 0.23). There were no other main or interaction effects in this group.

Dose–response associations between recent stress and BDNF in depression with comorbid anxiety

To evaluate whether there was a dose–response association between the number of reported recent life events and BDNF concentrations, an additional ancova was conducted on the three categories of recent stress: no (n = 232) versus one recent life event (n = 92) versus two or more life events (n = 78), which confirmed the effect of recent stress ($F_{2, 388}$ = 3.17, P = .04). However, post hoc comparisons showed that BDNF was not affected in a dose-dependent way: whereas individuals reporting one life event had lower BDNF concentrations (8.19 ± 3.09) than those reporting no life event (9.11 ± 3.06; P = .02; d = 0.30), the group reporting two or more life events (8.53 ± 3.35) did not differ from the group reporting no or one life event (both P-values > .10).

Dose–response associations between childhood abuse and BDNF in depression with comorbid anxiety

To investigate a dose–response association between childhood abuse and BDNF concentrations in met carriers versus the homozygous val group, a 3 (no childhood abuse [n = 225], one type of childhood abuse [n = 81], or two or more types of childhood abuse [n = 96]) times 2 (val^{66}met: homozygous val/val versus met carriers) Ancova was conducted (**Figure 1 ↓**). An interaction was found between childhood abuse categories and val^{66}met ($F_{3, 383}$ = 6.47, P < .0001), indicating that BDNF concentrations decrease in met carriers with an increasing umber of types of childhood abuse exposure ($F_{3, 132}$ = 5.00, P = .003), while in the homozygous val carriers, no main effect of childhood abuse categories was found (P = .11). Post-hoc comparisons showed that BDNF concentrations were low in met carriers when participants reported two or more types of childhood abuse compared to no childhood abuse (P = .001, d = 0.63), but not when they reported one type of childhood abuse (P = .11). Moreover, met carriers had lower BDNF concentrations when reporting two or more types of CA compared to the val/val group that reported two or more types of childhood abuse (P = .006, d = 0.66), but not when reporting one type of childhood abuse (P = .46). Furthermore, a main effect of val^{66}met also emerged ($F_{3, 383}$ = 5.56, P = .02), with met carriers having lower BDNF concentrations compared to val/val individuals, regardless of childhood abuse categories.

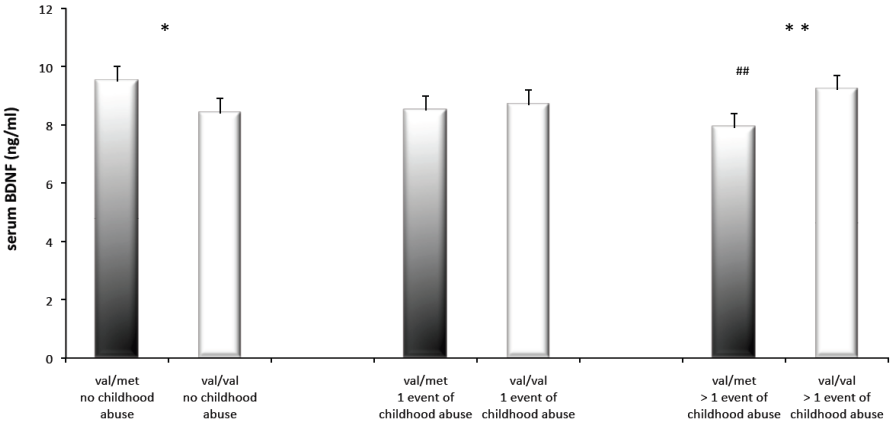

Figure 1. Mean serum BDNF concentrations by exposure to the number of childhood abuse types and val^{66}met. Error bars reflect the *SEM*. * denotes statistical significance at $P < .05$; ** denotes statistical significance at $P < .01$; ## denotes statistical significance at $P < .01$ when comparing the val/met group with 2 or more types of childhood abuse with the val/met group no childhood abuse

Depression with comorbid anxiety

The results of the same ancova in the depression with comorbid anxiety group showed a main effect of recent stress on BDNF concentrations ($F_{1,\ 1,014} = 4.14$, $P = .04$, $d = 0.16$). In contrast to the findings in the MDD − anxiety group, recent stress exposure was associated with elevated BDNF concentrations (9.26 ± 3.8) compared to not being exposed to recent stressful events (8.47 ± 3.4). Moreover, recent stress tended to interact with val^{66}met ($F_{1,\ 1,014} = 3.04$, $P = .08$). No other main or interaction effects were significant in the depressed with comorbid anxiety group.

Dose–response associations between recent stress and BDNF in depression with comorbid anxiety

To evaluate whether there was a dose–response association between reported life events in the val/met versus the val/val, an additional 3 (categories of recent stress: no [$n = 606$], one recent life event [$n = 246$], or two or more life events [$n = 181$]) times 2 (BDNF val^{66}met: val/val versus met carriers) anova was conducted. Here, only a trend for life events categories was found ($F_{2,\ 1,016} = 2.33$, $P = .09$) and no interaction with val^{66}met ($P = .29$). When comparing the means for the three recent stress groups post hoc, only individuals reporting one life event (9.58 ± 4.00) had higher BDNF concentrations than those reporting no life events (8.98 ± 3.42; $P = .03$), whereas the group reporting two or more life events (9.14 ± 3.42) did not differ from the group reporting 1 or no life event (both P's > .10).

DISCUSSION

The main new result of this study is that the impact of childhood abuse on serum BDNF concentrations appears to be dependent on variations on the BDNF val^{66}met polymorphism, at least in individuals with (lifetime) depression without co-morbid anxiety. In BDNF met carriers, exposure to childhood abuse was associated with reduced serum concentrations of BDNF, and these differences were most pronounced in met carriers who also reported negative life event(s) in the past year. In addition, these BDNF reductions associated with childhood abuse were linear in nature, so that BDNF concentrations were lowest in met carriers reporting two or more types of childhood abuse. Moreover, these associations were not accounted for by the presence of a current depression or by other potentially confounding factors, such as gender or the use of an antidepressant, as these factors did not differ between the homozygous val/val and the met carriers. The val/val group, on the other hand, did not show reductions in BDNF concentrations related to childhood abuse, and in the depressed group without co-morbid anxiety, BDNF concentrations were even higher in val/val participants reporting childhood abuse. Taken together, these findings are in line with the idea that met carriers are more sensitive to stress induced down-regulation of BDNF.

A second main finding is that exposure to stressful events that occurred in the past year reduced BDNF

concentrations, independent of variation on the val^{66}met polymorphism, at least in the depressed group without co-morbid anxiety. These results extend the finding of two previous studies showing lowered BDNF concentrations in bipolar patients reporting negative life events (Kauer-Sant'Anna *et al*., 2007), and in women with high risk of depression reporting recent life events (Trajkovksa *et al*., 2008). In the group with co-morbid anxiety, recent stress exposure was associated with increased BDNF concentrations, however. Moreover, while childhood abuse was associated with reduced BDNF in the met carriers, BDNF concentrations were not associated with childhood abuse in the co-morbid group. These findings seem to suggest that a co-morbid anxiety disorder may counteract the down-regulation of BDNF associated with childhood abuse and recent stressful events. So far, it is unclear how this relates to the symptomatology of depression with and without co-morbid anxiety, particularly because childhood abuse has specifically been linked to co-morbidity of depression and anxiety disorders (Hovens *et al*., 2009). Moreover, some studies have associated the val/val polymorphism with anxiety, rather than with depression (Gatt *et al*., 2009), but in the group with co-morbid anxiety disorders, we did not find any indications that val/val individuals have lower BDNF concentrations.

Although it should be taken into account that this is a cross-sectional study, preventing causal inferences about the impact of childhood abuse, it is remarkable that exposure to childhood abuse, which occurred in many individuals more than 25 years ago, is associated with decreased BDNF concentrations, at least in met carriers without co-morbid anxiety disorders. These reductions of serum BDNF concentrations in individuals with reported childhood abuse suggest that exposure to chronic stress during childhood may lead to a long-lasting down-regulation of the neurotrophic system, which might be further reduced by recent stressful events. These results extend the findings of previous studies showing lower concentrations of BDNF in patients with current depression and a history of childhood abuse (Grassi-Oliveira *et al*., 2008). Since variation at the val^{66}met locus were not taken into account in this study, it remains unclear whether the effects of childhood abuse were mainly driven by met carriers, as might be expected on the basis of our results.

The finding of reduced BDNF concentrations in met carriers is also of interest when considered in the context of previous findings, indicating that met carriers are particularly vulnerable to the impact of childhood abuse with respect to depressive symptoms (Kaufman *et al*., 2006; Wichers *et al*., 2008) and enhanced loss of hippocampal prefrontal gray matter (Gatt *et al*., 2009), given that low BDNF concentrations are associated with depression (Sen *et al*. 2008). Moreover, reductions in BDNF expression can have a direct impact on neuronal growth and plasticity in fronto-hippocampal networks (Murakami *et al*., 2005; Song *et al*., 2006). It should be noted that, in our sample, no associations were found between met carriers and higher depression severity after childhood abuse, however, which is consistent with some recent studies (Aguilera *et al*., 2009; Nederhof *et al*., 2010). Definitely, longitudinal studies are needed to further unravel the developmental trajectories relating exposure to childhood abuse and recent life events to low BDNF concentrations and altered brain structures and functioning.

One other interesting observation is that variations on the BDNF val^{66}met polymorphism itself were not directly associated with variations in BDNF concentrations even though, among individuals reporting no childhood abuse, met carriers had higher BDNF concentrations compared to homozygous val carriers. Very few studies in humans investigated the association between the val^{66}met and serum BDNF concentrations. One study in psychological healthy individuals also reported enhanced serum BDNF concentrations in met carriers compared to val/val individuals (Lang *et al*., 2009). Two other studies did not find an association between the val^{66}met polymorphism and variations in peripheral BDNF concentrations, not in a sample of depressed patients (Duncan *et al*., 2009) nor among healthy twins (Vinberg *et al*., 2009).

Taken together, findings regarding associations between the val^{66}met polymorphism and variations in

peripheral BDNF concentrations in humans are mixed. This could be due to the fact that, in previous studies, neither childhood abuse nor exposure to recent stressful events has been taken into account. Furthermore, this might also be related to the fact that the direct associations between val^{66}met and BDNF concentrations in blood serum, if anything, appear to be rather small in individuals reporting no childhood abuse (in our study d = 0.19) and thus can only be detected in large samples. In sum, this study has shown that childhood abuse is associated with reduced BDNF concentrations in met carriers with lifetime depression (without comorbid anxiety), whereas serum BDNF concentrations of val/val carriers do not seem to be affected by exposure to childhood abuse.

A number of limitations should be taken into account when evaluating these findings. First, the reliability of participants' recall of events from childhood may vary, given the long time gap between occurrence and recall. Self-reported childhood abuse requires caution when interpreting the results, although Goodman *et al*. (1999) observed good reliability among psychiatrically ill women. Related to this, one cannot rule out that the association between childhood abuse or recent stress and low BDNF concentrations in individuals with lifetime depression could (in part) be spurious, in the sense that individuals with a current depressed mood might have low BDNF concentrations and also experience life events in a (more) negative way, without these factors being directly related to each other. We do not consider this possibility very likely, however. First of all, we have previously shown that the association between negative life events and depression is independent of current mood state (Spinhoven *et al*., 2010). Moreover, in all analyses we added current versus remitted depression as a covariate, and the associations between life events and BDNF remain statistically significant when taking current mood state into account. A longitudinal design would be optimal to assess whether pre-differences versus post differences in serum BDNF are directly affected by stressful events. Another limitation is that we assessed serum BDNF concentrations, which may not be a direct measure of central BDNF, even though previous studies in animals showed that BDNF can cross the blood–brain barrier in both directions (Pan *et al*., 1998) and a strong association has been reported between central and peripheral BDNF concentrations (Karege *et al*., 2002). A third limitation is that we only assessed the val^{66}met variant, whereas there are more loci on the BDNF gene that might be associated with variations in serum BDNF concentrations. Moreover, gene-gene interactions, in particular interactions with the 5HTT polymorphism, were not addressed in this study, whereas these interactions have been shown to be relevant in predicting depression in combination with childhood abuse in some studies (*e.g.,* Kaufman *et al*., 2006; Wichers *et al*., 2008), although not in others (Gatt *et al*., 2009).

Despite the considerations mentioned above, we provide new and important evidence to suggest that a chain of events, commencing with gene-environment interactions and their impact on (set points of) BDNF, may lead to low BDNF concentrations in patients with lifetime depressive disorders.

CHAPTER 8

BDNF val^{66}met affects hippocampal volume and emotion-related hippocampal memory activity

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SIGNIFICANCE: In this study we further the understanding of the association of BDNF val^{66}met with hippocampal volume/functioning and cognitive performance. Critically, we take trauma/stress exposure into account. We find a small effect of val^{66}met on hippocampal volume and that trauma exposure in childhood accounts for individual differences in hippocampal encoding activity. This latter effect seems to manifests itself differently as a function of val^{66}met. These findings, although in need for replication, raise the question whether met carriers show abnormal brain response on emotional laden stimuli. This message comes with the notion that again, no effects no behavioral effects were observed alongside the neurobiological differences.

ABSTRACT

The val^{66}met polymorphism on the BDNF gene has been reported to explain individual differences in hippocampal volume and memory related activity. These findings, however, have not been replicated consistently and no studies to date controlled for the potentially confounding impact of early life stress, such as childhood abuse, and psychiatric status. Using structural and functional MRI we therefore investigated in 126 depressed and/or anxious patients and 31 healthy control subjects the effects of val^{66}met on hippocampal volume and encoding activity of emotional laden and neutral words, while taking into account childhood abuse and psychiatric status. Our results show slightly lower hippocampal volumes in carriers of a met allele (n = 54) relative to val/val homozygotes (n = 103; P = 0.02, Cohen's d = 0.37), which appeared to be independent of childhood abuse and psychiatric status. For hippocampal encoding activity we found a val^{66}met–word valence interaction (P = 0.02) such that carriers of a met allele showed increased levels of activity in response to negative words. This, however, was only evident in the absence of childhood abuse, as abused val/val homozygotes showed hippocampal encoding activity for negative words that was comparable to that of carriers of a met allele. Neither psychiatric status nor memory accuracy did account for these associations. In conclusion, BDNF val^{66}met appears to have a small, yet significant, impact on hippocampal volume independently of childhood abuse and psychiatric status. Furthermore, early adverse experiences such as childhood abuse account for individual differences in hippocampal encoding activity of negative stimuli but this effect apparently manifests differently as a function of val^{66}met.

INTRODUCTION

Brain-Derived Neurotrophic Factor (BDNF) regulates the sprouting of axons and dendrites in the hippocampus, a key structure for emotion and memory processing (Murer *et al*., 2001; Komulainen *et al*., 2008). Rodent studies, for example, have shown that BDNF modulates hippocampal neuronal differentiation (Taliaz *et al*., 2010) and hippocampal dependent memory (Choi *et al*., 2010). Moreover, human studies have reported a positive relation between BDNF concentrations, hippocampal volume, and memory performance (Gunstad *et al*., 2008; Erickson *et al*., 2010).

Studies focusing on a single nucleotide site in the DNA sequence of the BDNF gene; val^{66}met (a valine [val] to methionine [met] insertion at codon 66) have partly confirmed the associations of BDNF protein expression with neurobiological and behavioral abnormalities. Egan and colleagues (2003) showed *in vitro* that the met allele is linked to a reduced activity-dependent expression of BDNF in hippocampal neurons of rats, a finding that was replicated by Chen *et al.* (2004). In addition, studies have shown that in the hippocampus the met allele is associated with diminished levels of N-acetyl-aspartate, a putative marker for neuronal integrity (Stern *et al*., 2008). In line with these findings, some studies have shown that the met allele is associated with impaired episodic memory (Egan *et al*., 2003) and executive functioning (Rybakowski *et al*., 2003; Erickson *et al*., 2008). Structural and functional Magnetic Resonance Imaging (MRI) studies further suggest that carriers of a met allele have smaller hippocampal volumes relative to val/val homozygotes (Pezawas *et al*., 2004; Frodl *et al*., 2007) and altered hippocampal activity during the encoding of stimuli (Egan *et al*., 2003).

Nevertheless, these findings have not been consistently replicated (Schofield *et al*., 2009; Benjamin *et al*., 2010; Lau *et al*., 2010; Gerritsen *et al*., 2012) which might be due to the inclusion of small samples and task characteristics such as the emotional valence of the stimuli. Furthermore, the occurrence of early trauma such as childhood abuse and psychiatric status represent sources of variation in hippocampal volume and function (reviewed in Bremner *et al*., 2007; MacQueen and Frodl, 2011) that have not been taken into account in previous studies. In addition, gene-environment interactions have been reported between BDNF val^{66}met and abuse on brain structure and activity (Gatt *et al*, 2009; Juhasz *et al*., 2010). As a consequence, the earlier reported associations between BDNF val^{66}met and hippocampal structure and function might be (partly) dependent on a history of childhood abuse or on psychiatric status.

The goal of this study, then, was to evaluate the effects of val^{66}met on hippocampal volume and on encoding related hippocampal activity while taking into account the potential influence of childhood abuse and diagnostic status. Given earlier conflicting findings we further aimed to extend previous findings by examining the effects of neutral, positive, and negative emotional stimuli on hippocampal activity.

MATERIAL AND METHODS

Subjects

The data analyzed are from the imaging sample of the Netherlands Study of Depression and Anxiety (NESDA; Penninx *et al*., 2008; van Tol *et al*., 2010). Included in the sample were 301 subjects of whom 233 were patients with a current depressive and/or anxiety disorder and 68 healthy control subjects. Genetic and high-quality functional and structural MRI data were available for 157 persons of whom 126 were depressed and/or anxious patients and 31 were healthy controls. Subjects in the current study did not differ from subjects in the NESDA imaging sample (N = 301) with regard to age (P = .98), gender (P = .22), and current diagnosis (P = .07).

Subjects underwent imaging at three different locations in the Netherlands: Academic Medical Center (AMC), University of Amsterdam, University Medical Center Groningen (UMCG), and Leiden University Medical Center (LUMC). To be eligible subjects had to be between 18 to 57 years of age and fluent in Dutch.

Exclusion criteria were having an Axis-I disorder other than a depressive and/or anxiety disorder (Diagnostic and Statistical Manual of Mental disorders fourth edition (DSM-IV; APA 1994), being on antidepressant treatment other than SSRIs at a stable dose (WHO 2008), a history of a major internal or neurological disorder, dependency on alcohol and/or drugs, smoking > 5 cigarettes a day, or hypertension (> 180/130 mmHG). The protocol and procedures were approved by each of the Ethical Committees of participating institutes and all subjects signed an informed consent.

Diagnoses of depressive and anxiety disorders were established according to the criteria set forth in the DSM-IV (APA, 1994) on the basis of responses to the Composite International Diagnostic Interview 2.1 (CIDI) lifetime version (WIttchen *et al*., 1991), a reliable and validated diagnostic tool (Wacker *et al*., 2006). The severity of depressive and anxiety symptoms was assessed with the Montgomery Åsberg Depression Rating Scale (MÅDRS; Montgomery and Åsberg; 1979) and the Beck Anxiety Inventory (BAI; Beck *et al*., 1988;) which both have been shown to have excellent psychometric characteristics (Davidson *et al*., 1986; Kabacoff *et al*., 1997).

Childhood abuse was assessed retrospectively using a semi-structured childhood trauma interview de Graaf *et al*., 2004 a, b). In this interview, participants were asked whether they had experienced emotional neglect or psychological abuse, physical abuse, and/or sexual abuse before the age of 16 years. After an affirmative answer, subjects were asked for details on the frequency of the events. Based on the sum and the frequency of abusive events an index (range 0-8) was calculated for each subject (for details see Wiersma *et al*., 2009).

Genotyping

For a detailed description of the procedures we refer to Boomsma *et al*. (2008). In sum, variation at the val^{66}met locus was extracted from whole-genome data using PLINK software version 1.07 (www.pngu.mgh.harvard.edu). In our sample, 103 subjects were val/val homozygotes (65.6%) and 54 subjects carried a met allele (34.4%). Two subjects (1.3%) with the met/met genotype were merged with heterozygous subjects into a group of met allele carriers. Genotype counts were 82 val^{66}val, 42 val^{66}met, and 2 met^{66}met in the patient group and 21 val^{66}val, 10 val^{66}met, and 0 met^{66}met in the healthy control group. Patient and healthy control samples did not differ with regard to genotype distribution (P = .77). Allele frequencies were in Hardy-Weinberg equilibrium in the GAIN-MDD sample in which the genotyping was performed (N = 3,530, χ^2_1 = 0.62, P = .43) and in the sub-sample on which we present data (n = 157, χ^2_1 = 2.66, P = .10).

Memory Paradigm

In the scanner subjects performed a subject-paced, event-related encoding task, similar to the paradigm described by Daselaar *et al*. (2003) and known to reliably activate the hippocampus. The task is described in detail elsewhere (van Tol *et al*., 2011). Briefly, during the encoding phase of the task 120 words (40 of neutral valence, 40 of positive valence, and 40 of negative valence) were presented in pseudo-randomized order. Subjects were instructed to classify these words according to valence. After a 10-minute retention interval, subjects were asked to complete a word recognition task. Subjects were instructed to indicate whether they had seen the word or whether the word was new. Discriminant accuracy was calculated as the proportion correctly recognized words minus the proportion false alarms (van Tol *et al*., 2011).

Image acquisition and data handling

Image acquisition and data handling are detailed elsewhere (van Tol *et al*., 2011, 2012). In sum, imaging data were collected using Philips 3-Tesla MRI scanners (Best, The Netherlands) using SENSE-6 and 8 channel

head coils (AMC and UMCG/LUMC respectively). Echo-planar images were obtained using a T2*-weighted gradient echo sequence with repetition time 2300 ms, a 30 ms echo time (UMCG 28ms), a matrix size of 96 × 96 (UMCG 64 × 64), producing 35 axial slices of 3 mm thickness direction interleaved, 2.29 × 2.29 mm in-plain resolution (UMCG 3 × 3). Anatomical imaging included a sagittal 3-D gradient-echo T1-weighted sequence with a repetition time of 9 ms and a 3.5 ms echo time producing slices with a voxel size of 1 × 1 × 1 mm. Imaging data were preprocessed with SPM5 (Statistic Parametric Mapping, London, UK; www.fil.ion.ucl.ac.uk/).

Preprocessing of the data included reorientation of the functional images to the anterior commissure, slice time correction, image realignment, registration of the T1-scan to the mean image, warping to Montreal Neurological Institute (MNI) space as defined by the SPM5 T1-template, reslicing to 3 × 3 × 3mm voxels, and spatial smoothing using an 8 mm FWHM Gaussian kernel. Haemodynamic responses to each stimulus were modeled with a delta function convolved with a synthetic haemodynamic response function and modulated using response times.

Contrast images for *subsequent hits* versus *baseline* were calculated for the neutral, positive, and negative word condition per subject on a voxel-by-voxel basis, based on subsequent recognition success and entered in a 2 (group: val/val homozygotes versus met carriers; independent factor) by 3 (condition: neutral, positive, negative (> baseline); dependent factor) Mancova with age, education and scan center as covariates. Mean BOLD signal change during successful encoding in the left and right hippocampus was extracted per condition (neutral/positive/neutral > baseline) using the MARSBAR toolbox (Brett *et al*., 2002). The hippocampal masks of the Automated Anatomical Labeling software package, implemented in the WFU Pick Atlas toolbox (Maldjian *et al*., 2003) were used to define the left and right hippocampal region.

Anatomical images were processed using an optimized Voxel Based Morphometry approach, following the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL; Ashburner, 2007) using SPM5 software implemented in Matlab 7.1.0 (The MathWorks Inc., Natick, MA, USA). For details see van Tol *et al*. (2011, 2012). To test for differences in regional brain volume, an independent samples *t*-test was set up for a voxel-wise comparison of the grey matter density images of the val/val homozygotes and met carriers, with age, scan center and total gray matter volume as covariates. Following a similar approach as for signal change extraction, the mean volume of the left and right hippocampus was additionally extracted. Data were exported to SPSS 18.0 (Chicago, IL, USA) for further analysis.

Statistical analyses

Computations were performed in SPSS 18.0. A *P* value of < .05 (2-tailed) was considered as the threshold for statistical significance. Demographical and clinical characteristics between groups were compared using Student's *t*-tests for continuous- and χ^2-tests for categorical data.

Main effects of val^{66}met on right, left, and total hippocampal volume were calculated using a Repeated Measures (RM) Ancova with left versus right hippocampal volume as the within-subjects factor and age, gender, number of years of education, SSRI use, alcohol use, scan site, and total gray matter volume as covariates. Ancova's were used to assess the effects of val^{66}met on memory accuracy and hippocampal activity during the encoding of neutral, positive, and negative words. To address val^{66}met–valence interaction effects on memory accuracy and hippocampal encoding activity we ran RM Ancova's with word valence (positive versus neutral and negative versus neutral) as within-subject factor and age, gender, number of years of education, SSRI use, alcohol use, scan site, hippocampal volume, memory accuracy, and handedness as covariates. If indicated by between-group differences in memory accuracy, accuracy scores were included as covariates in the analyses on hippocampal encoding activity.

Possible interaction effects of val^{66}met with abuse and diagnosis (dummy variables coding for healthy, depressed, depressed-anxious, and anxious) on hippocampal volume, memory accuracy, and hippocampal encoding activity were evaluated using hierarchical stepwise regression analyses if indicated by statistically significant associations in the above described analyses. Regression analyses consisted of three steps: (**I**) covariates, (**II**) val^{66}met, childhood abuse, and diagnosis, and (**III**) the interaction terms val^{66}met × abuse and val^{66}met × diagnosis. Analyses were rerun with lifetime instead of current diagnosis. Tolerance of the predictors and normality of error variances were verified.

To assess regional specificity of val^{66}met within the hippocampus, voxel-wise analyses were repeated on the gray matter density maps and contrast maps reflecting encoding related activity using SPM5, with the threshold set at $P < .001$, uncorrected. For regions outside the hippocampus, a threshold of $P < .05$, FWE corrected was set.

RESULTS

The overall sample ($N = 157$) had a mean age of 37.39 ± 10.08 years and included 100 women (63.7%). Demographical and clinical characteristics of the sample are given in **Table 1 ↓** by BDNF genotype. There were no statistically significant differences between the genotype groups in terms of demographical and clinical variables. Furthermore, val^{66}met was not differentially associated with exposure to childhood abuse (dichotomous nor with exposure to the specific types of childhood abuse (all *P*-values > .75).

Table 1. Demographic and clinical characteristics (mean ± *STD* or percentages) by BDNF genotype

		val^{66}val (n = 103)	val^{66}met (n = 54)	*P*-value
Females	%	64.1	63.0	= .89
Age		37.1 ± 10.0	37.9 ± 10.4	= .65
Education (years)		12.4 ± 3.0	12.6 ± 3.3	= .70
Smoker	%	33.0	23.2	= .14
Alcohol use	%	56.2	60.0	= .58
SSRI use	%	30.1	20.4	= .19
Right handed	%	91.3	94.4	= .48
Childhood trauma index range 0 -8)		1.6 ± 2.0	1.7 ± 2.3	= .87
Diagnostic status				= .78 [1]
Healthy controls	%	20.4	18.5	= .78
Depression	%	22.3	25.9	= .61
Anxiety [2]	%	20.4	14.8	= .39
Depression and anxiety [2]	%	20.4	24.1	= .59
Depression severity, MÅDRS		11.6 ± 8.8	13.6 ± 11.6	= .23
Anxiety severity, BAI		11.7 ± 9.1	13.3 ± 11.2	= .84

Abbreviations: BAI, Becks Anxiety Inventory; MADRS, Montgomery Åsberg Depression Rating Scale; SSRI, Selective Serotonin Reuptake Inhibitor
[1] Chi-square test (3 *df*) for differences in distribution of the val and the met allele over diagnoses
[2] Included a diagnosis of social phobia, panic disorder, generalized anxiety disorder, and/or agoraphobia

BDNF val^{66}met and hippocampal volume

Total hippocampal volume was smaller in carriers of a met allele relative to val/val homozygotes ($F_{1,180} = 5.33$, $P = .02$; standardized Cohen's $d = 0.38$; see **Figure 1 ↓** and **Table 2 ↓** for covariate adjusted means on total, right, and left hippocampal volume ± Standard Error [*SE*]). No interaction of val^{66}met × right versus

left hippocampus was observed (P = .63). BDNF val^{66}met had no effect on total gray matter volume (P = .60). Voxelwise analyses of the hippocampus confirmed these findings, with the peak voxel located in the posterior part of the hippocampus (MNI coordinate: Right hippocampus: [x = 18 y = -33 z = 8 and x = 21 y = -30 z = -4], Z = 3.61/3.42, k = 29/17, $P_{FWE\text{-}ROI}$ = .018; Left hippocampus: [x = -18 y = -36 z = 8], Z = 3.17, k = 4, $P_{FWE\text{-}ROI}$ = .062).

Regression analyses were used to evaluate whether the smaller hippocampal volume in met carriers as compared to val/val homozygotes were moderated by the effects of abuse or diagnostic status. Main effects of childhood abuse and psychiatric status, and interaction effects of val^{66}met with childhood abuse and psychiatric status on hippocampal volume were not observed (all P's > .10). The main effect of val^{66}met remained statistically significant after the inclusion of childhood abuse and psychiatric status in the model (B = -0.13, 95% Confidence Interval [CI] = -0.24 to -0.02, P = .02). Similar results were obtained in analyses with lifetime instead of current diagnosis and in analysis in which continuous measures for childhood abuse and depression severity were included as predictors (data not shown). No effect of BDNF val^{66}met was observed on other structures at the set threshold (data not shown).

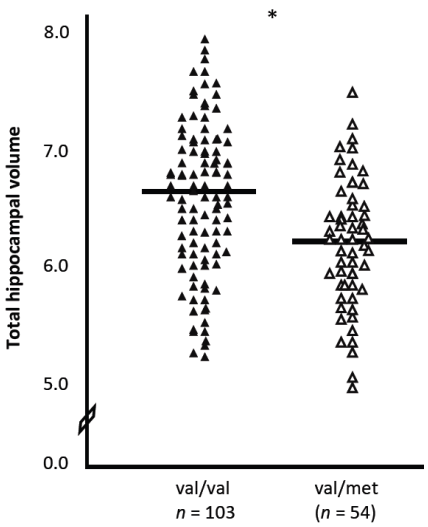

Figure 1. Hippocampal volume by val/val and val/met genotype. Data are covariate adjusted (see the method section).

* Denotes statistical significance at P < 0.05 (d = 0.38).

Table 2 Cerebral and hippocampal volumes and hippocampal related encoding activity (mean ± *SEM*) by BDNF genotype and word valence (neutral, positive, and negative)

	val^{66}val (n = 103)	val^{66}met (n = 54)	P value
Total grey matter volume [1]	736.57 ± 5.35	731.66 ± 7.45	= .60
Hippocampal volume [1, 2]			
Total	6.45 ± 0.03	6.31 ± 0.04	= .02
Right	3.06 ± 0.02	2.99 ± 0.02	= .01
Left	3.39 ± 0.02	3.31 ± 0.02	= .05
Hippocampal encoding activity [1, 3]			
Neutral words	0.15 ± 0.04	0.16 ± 0.06	= .97
Positive words	0.15 ± 0.05	0.23 ± 0.07	= .30
Negative words	0.20 ± 0.05	0.36 ± 0.06	= .04

[1] All mean values are corrected for gender, age, years of education, SSRI and alcohol use, and site of scanning
[2] Mean values are additionally corrected for total cerebral grey matter volume
[3] Mean values are additionally corrected for total hippocampal volume

BDNF val^{66}met and task performance

Persons who were val/val homozygotes did not differ from met carriers with regard to the discriminant accuracy of neutral, positive, and negative words (all *P's* > .35). There also were no overall differences in discriminant accuracy as a function of genotype (covariate adjusted means ± SE: val/val homozygotes = 0.57 ± 0.01 versus met carriers = 0.58 ± 0.02; *P* = .85). Interaction effects of val^{66}met and word valence on memory accuracy were not observed (all *P*'s > .10). Furthermore, memory accuracy was unrelated to hippocampal volume (Pearson's *r* = 0.13; *P* = .10) and to hippocampal encoding activity (*r* = 0.04; *P* = .66).

BDNF val^{66}met and hippocampal activity

Main effects of val^{66}met and word valence on hippocampal activity during the encoding of neutral and positive words were not observed (see **Table 2 ↑**). However, val^{66}met interacted with neutral versus negative word valence (*P* = .02) such that hippocampal activity was higher in carriers of a met allele in the negative word condition relative to hippocampal activity in the neutral word condition (Bonferroni corrected *P* = not significant). This was not observed in val/val homozygotes (see **Figure 2 ↓** and **Table 2 ↑** for covariate adjusted means ± *SE* by word valence). No val^{66}met-neutral versus positive word valence interaction effect on encoding activity was found (*P* = .17). Effects of lateralization were not observed. Voxel-wise analyses located the peak voxel of the interaction between negative versus neutral encoding times val^{66}met cluster at the left posterior hippocampus ([x = -21 y = -27 z = -6], $F_{1, 461}$ = 14,11, *Z* = 3.55, P_{FWE_ROI} = .024, K [number of voxels] = 15).

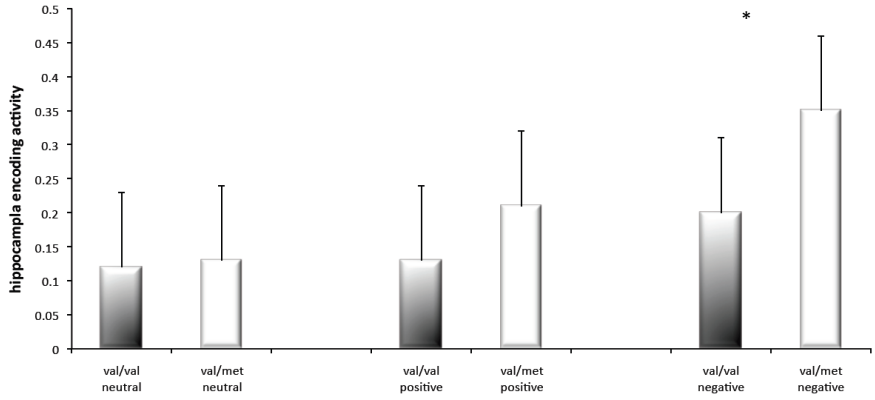

Figure 2. Plotted are covariate adjusted mean total hippocampal activity levels during encoding by stimulus valence and val^{66}met genotype (val/val *n* = 103; val/met *n* = 54). Error bars reflect the *SEM*.

* denotes statistical significance

Exploratory voxel-wise whole brain analyses showed no statistical significant effects of val^{66}met and val^{66}met-word valence interactions in brain areas other than the hippocampus at the a priori set threshold of *P* < .05, FWE corrected.

Regression analyses were used to evaluate whether the higher hippocampal activity during the encoding of negative words were moderated by the effects of abuse or diagnostic status. Hippocampal encoding activity in response to words of negative valence was higher in abused subjects as compared to non-abused subjects (*B* = 0.16, 95% CI = 0.05 to 0.28, *P* = .007). In addition we found a val^{66}met-childhood abuse interaction effect (*B* = -0.10, 95% CI = -0.17 to -0.02, *P* = .01) showing that childhood abuse predicted increased hippocampal activation in response to negative words in val/val homozygotes (*P* = .009) but not in carriers of a met allele (*P* = .34) (see **Figure 3 ↓**). Effects of psychiatric status (lifetime and current) and val^{66}met by psychiatric status interaction effects were not observed (all *P*'s > .10). Adding memory accuracy as a predictor to the model did not change our results (data not shown) making it unlikely that these results are accounted for by genotype differences regarding attention or effort.

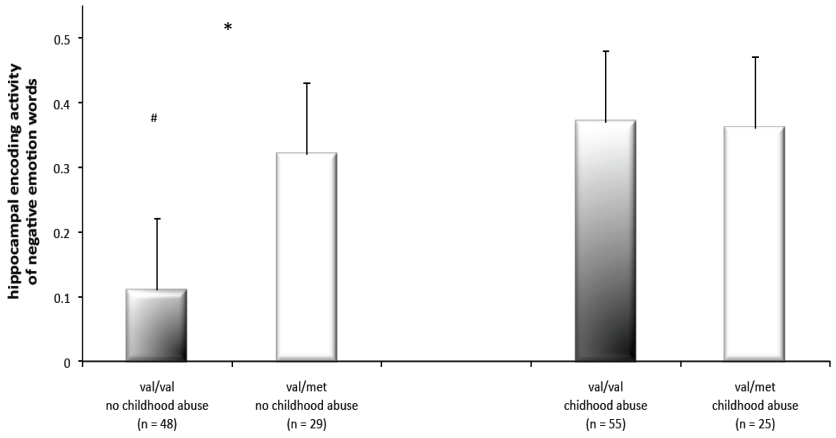

Figure 3. Plotted are covariate adjusted mean total hippocampal activity levels during the encoding of negative words by childhood abuse exposure and val^{66}met.
Error bars reflect the *SEM*.
The val^{66}met – childhood abuse interaction effect is statistically significant at P = .01.

* denotes statistical significance at P < .05. # denotes statistical significance at P < .05 for the difference between abused and non-abused val homozygotes

DISCUSSION

We addressed the effects of val^{66}met on hippocampal volume and function while taking into account the possible confounding effects of childhood abuse and psychiatric status.

In line with some previous studies (Szeszko *et al*., 2005; Bueller *et al*., 2006; Frodl *et al*., 2007) but not all (*e.g.,* Gerritsen *et al*., 2012) we find smaller hippocampal volumes in carriers of a met allele relative to val/val homozygotes. This effect has generally been explained by abnormal intracellular trafficking and impaired activity secretion of BDNF, and by extension aberrant (trophic support in carriers of a met allele relative to the val/val homozygotes that have been shown in *in vitro* experiments (Egan *et al*., 2003; Chen *et al*., 2004). But since atrophy of the hippocampus has also been associated with (early) stress and/or current or remitted depression (MacQueen and Frodl, 2011) it is crucial to exclude the possible confounding effects of these variables. Our data suggests that the association between the met allele and hippocampal volume is independent of childhood abuse. This finding is at odds with those of Gatt and colleagues (2009) who modeled the interaction of early life stress and val^{66}met in the prediction of hippocampal volume and found that the combination of carrying a met allele and being exposed to early life stress was associated with smaller hippocampal volumes in healthy adults. It could be that the observed discrepancy between the results of Gatt *et al*. (2009) and ours might be explained by a broader definition of early life stress by Gatt and colleagues (2009) who included for example also *illness and exposure to natural disasters* as stressful events whereas we focused on childhood abuse including physical, sexual, and emotional abuse. Furthermore, the study subjects of Gatt *et al*. (2009) were all healthy control subjects (N = 89) whereas we studied mostly patients. However, exactly how these differences between the studies could have led to a different pattern of results is unclear. In line with Frodl and colleagues (2007), we show that lifetime and current psychiatric status does not thrive the val^{66}met genotype effect on hippocampal volume, providing evidence for a direct association between the met allele and small hippocampal volume that further appears to be specific to the hippocampus.

In addition to reduced hippocampal volume, we show that val^{66}met interacts with word valence such that encoding activity is increased in carriers of a met allele during the negative word condition and not in the neutral or positive word condition. This effect was not observed in other brain areas than the hippocampus and is consistent with some studies in which emotional stimuli were used (*e.g.,* Dennis *et al*., 2010). We could not replicate the finding of higher hippocampal activation in carriers of a met allele in response to neutral stimuli that was reported in the seminal study by Egan *et al*. (2003). On the basis of a recent study that showed that negative affectivity increased more in response to social stress in met carriers as compared to val/val homozygotes (Wichers *et al*., 2008) one may speculate that carriers of a met

allele are more sensitive or reactive to negative stimuli. Owing to a possible relation between higher hippocampal activity and psychopathology (Thomaes *et al*., 2009) this finding might concur with studies that show a link between the met allele and depression (reviewed in Verhagen *et al*., 2010).

We further found, in line with some studies that childhood abuse predicts higher levels of hippocampal encoding activity (Werner *et al*., 2009; Heim *et al*., 2010). However, from our data it appears that childhood abuse is associated with a relative increase in hippocampal activity in val/val homozygotes only and not in carriers of a met allele. Although speculative, an interpretation may be that higher levels of hippocampal activity after exposure to childhood abuse in val/val homozygotes reflect a higher sensitivity for emotionally negative stimuli in that in carriers of a met allele is present regardless of exposure to childhood abuse. This idea is in line with studies that report hippocampal dysfunction in various severe psychiatric illnesses, particularly if exposure to childhood abuse is documented (Thomaes *et al*., 2009; Heim *et al*., 2010; MacQueen and Frodl, 2011).

Despite differences in hippocampal volume and activity between val/val homozygotes and carriers of a met allele we did not find differences in memory accuracy and clinical variables (e.g., depression severity) as a function of BDNF genotype. This may suggest that our findings are relevant for both healthy individuals and patients and also is pertinent to the debate on the relationship between hippocampal volume and function with behavioral performance. However, with regard to the absence of associations between hippocampal volume, hippocampal function, and memory performance, a recent review on 80 studies showed, in line with our findings, that the model: *a bigger brain structure → greater brain response → better performance* may not reflect reality (Eyler *et al*., 2010).

A notable strength of our study is that the findings are derived from a genetically homogeneous sample making it unlikely that our results are devoid by population stratification (Cardon and Palmer, 2003). Furthermore, we studied the effects of val^{66}met in the context of childhood abuse and emotional valence of stimuli, and our results clearly highlight the importance of including such variables. A few weaknesses of our study also merit attention. Obviously, we cannot exclude the possibility that other polymorphisms on the BDNF gene or on other genes, notably those that constitute the neurotrophic pathway (e.g., CREB1 and NTRK2; see for example Juhasz *et al*., 2010) might have contributed to the effects that we observed. With regard to our self-reported measurement of childhood abuse it should be noted that the validity and reliability of recall might vary by diagnosis and time since abuse took place. Furthermore, in the face of negative findings statistical power is important to take into account. Overall we had a comparatively large sample size, but our analysis on psychiatric status might have been underpowered because patient samples may have been too small (*e.g.,* only 31 healthy control subjects) to detect main effect sizes that are reported to be moderate at best (Campbell *et al*., 2004; Videbech *et al*., 2004). Finally, although we speculate that carriers of a met allele are more reactive to emotionally negative laden stimuli as compared to val/val homozygotes we are not able to confirm this because we have no subjective ratings of the stimuli by our participants.

In sum, our results suggest that BDNF val^{66}met has a small effect on hippocampal volume and this effect appears to be independent of childhood abuse and psychiatric status. Furthermore, gene-environment interactions between val^{66}met and childhood abuse account for individual differences in hippocampal encoding activity of negative stimuli. Important venues for future research are to delineate the exact mechanisms, *in vivo*, through which the met allele produces its effect on hippocampal volume and function. In addition, it remains to be investigated, in longitudinal designs, whether or not the effects of val^{66}met on hippocampal volume and activity are predictive for individual cognitive functioning and psychological wellbeing.

CHAPTER 9

A systematic review on the association between val^{66}met and hippocampal volume – a genuine effect or a winners curse?

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SIGNIFICANCE: Over the years, the finding that met carriers at the BDNF val^{66}met locus have a relatively low hippocampal volume is a pillar under the neurotrophinn hypothesis to which basically all paper on the genotype refer (total number of citations for the first paper on this issue [Pezawas *et al.,* 2005] is > 500). Here however we show that this association probably is non-existent and due to underpowered studies.

ABSTRACT

Inconsistenties have been reported with regard to an association between val^{66}met, a single nucleotide polymorphism on the BDNF gene, and hippocampal volume. We performed a systematic review and a meta-analysis to determine the magnitude and direction of this putative association and estimated the potential influence of demographical, clinical, and methodological characteristics of studies. Tests of publication bias and time-related trends were performed and statistical power of the included studies was calculated. The literature search for MRI studies on differences in total hippocampal volume as a function of BDNF val^{66}met returned twenty-five records that fulfilled our criteria (total N = 3,620). Meta-analysis showed that carriers of a met allele had lower hippocampal volumes relative to val/val homozygotes (d = 0.13, P = .02). Between-study heterogeneity in effect size estimates was substantial and this could not be explained by demographical, clinical, and methodological differences across studies. Funnel plot inspection and trim-and-fill estimations suggested evidence for publication bias and effect sizes decreased substantially over the years (Pearson's r = -0.54, P < .01). All included studies were underpowered. This meta-analysis suggests that carriers of a met allele have lower total hippocampal volumes relative to val/val homozygotes. However, the effect sizes on this association converged closer to null with virtually each attempt at replication and were based on underpowered studies. Together our findings may suggest that the reported association between BDNF val^{66}met and hippocampal volume is not a genuine biological effect of the met allele but likely represents a winners-curse.

INTRODUCTION

Brain-Derived Neurotrophic Factor (BDNF) has been implicated in the pathophysiology of major depressive and bipolar disorder, and schizophrenia (Krishnan and Nestler, 2010). BDNF is a neuronal growth factor that has an array of functions including the induction of neuronal sprouting and differentiation (Poo, 2001). The role of BDNF is particularly evident in the hippocampus where it regulates processes such as learning (Lu and Gottschalk, 2000; Tapia-Arancibia *et al.,* 2008). Besides, by acting on hippocampal networks, BDNF is believed to be a moderator of mood (Taliaz *et al.,* 2009).

An intriguing feature of the expression of BDNF is that it is, unlike other neurotrophins, not only secreted constitutively but also in response to neuronal activity (*i.e.,* activity dependent secretion; Egan *et al.,* 2003). Interestingly, *in vitro* experiments have shown that the activity dependent secretion of BDNF is dependent on a single nucleotide site on the BDNF gene; val^{66}met, a valine into methionine insertion at codon 66 (Egan *et al.,* 2003; Chen *et al.,* 2004). Congruent with this is the finding that transgenic mice that carried a met allele had less dendritic complexity in the hippocampus and a reduced hippocampal volume (Magarinos *et al.,* 2010). Furthermore, in *in vitro* experiments the met allele has been linked to diminished neuronal integrity (Egan *et al.,* 2003; Stern *et al.,* 2010). Finally, human data are suggestive for an association between the met allele and the incidence of mood disorders (Verhagen *et al.,* 2008), schizophrenia (Gratacos *et al.,* 2007), and bipolar disorder (Rakofsky *et al.,* 2011). Taken together, these data add considerably to the idea that BDNF expression contributes to psychopathological characteristics and that this might be mediated by variation at the val^{66}met locus (for a critical review see Groves 2007).

In line with this idea, two high impact papers (Pezawas *et al.,* 2004; Szeszko *et al.,* 2005), using Magnetic Resonance Imaging (MRI) techniques, showed lower hippocampal volumes in carriers of a met allele relative to val/val homozygotes. This message inspired many and the association between val^{66}met and hippocampal volume became an area of interest, not at least because the hippocampus is considered to be a clinically relevant phenotype (MacQueen and Frodl, 2011). However, subsequent data with regard to this association is heterogeneous (*e.g.,* Dutt *et al.,* 2009). Reasons for this might be that the effect of val^{66}met on hippocampal volume is small and that therefore some studies may have lacked the necessary statistical power to detect it (*i.e.,* false negatives) or that the two pioneering papers may have overestimated the true effect (*i.e.,* a winners curse). Alternatively it could be that heterogeneity in findings is caused by demographical, clinical, or methodological differences across studies.

When faced with non-uniform findings it is useful to aggregate data over studies in order to learn about the most plausible nature of an association (Lohmueller *et al.,* 2003). Hence, we determined the magnitude of the putative association between BDNF val^{66}met and total hippocampal volume by means of a meta-analysis. The potential moderating influence of demographical, clinical, and methodological characteristics of studies were also tested and tests of publication bias were performed.

METHOD

Search Strategy

Using the terms: val^{66}met OR rs6265 AND hippocampus and val^{66}met OR rs6265 AND hippocampal volume two of us (BB and MM) searched the database PUBMED (www.ncbi.nlm.nih) through February 1st 2012 for human MRI studies on differences in hippocampal volume as a function of BDNF val^{66}met. The digital search was supplemented by a backward search in which all the references that were made to the 2 seminal papers were screened and by examining the reference sections of the retrieved records. We selected for inclusion human MRI studies that reported on differences in total hippocampal volume between val/val homozygotes and carriers of a met allele. Inclusion was independent of demographic (*e.g.,* gender), clinical (*e.g.,* diagnostic status), and methodological characteristics (*e.g.,* Voxel-Based Morphology

[VBM] or actual volume measurements) of the sample and the study. Our search yielded 81 papers of which 25 records (*k*) fulfilled our inclusion criteria (total *N* = 3,620). For detailed information on the search strategy and the results of this strategy we refer to the flow chart (**Figure 1 ↓**).

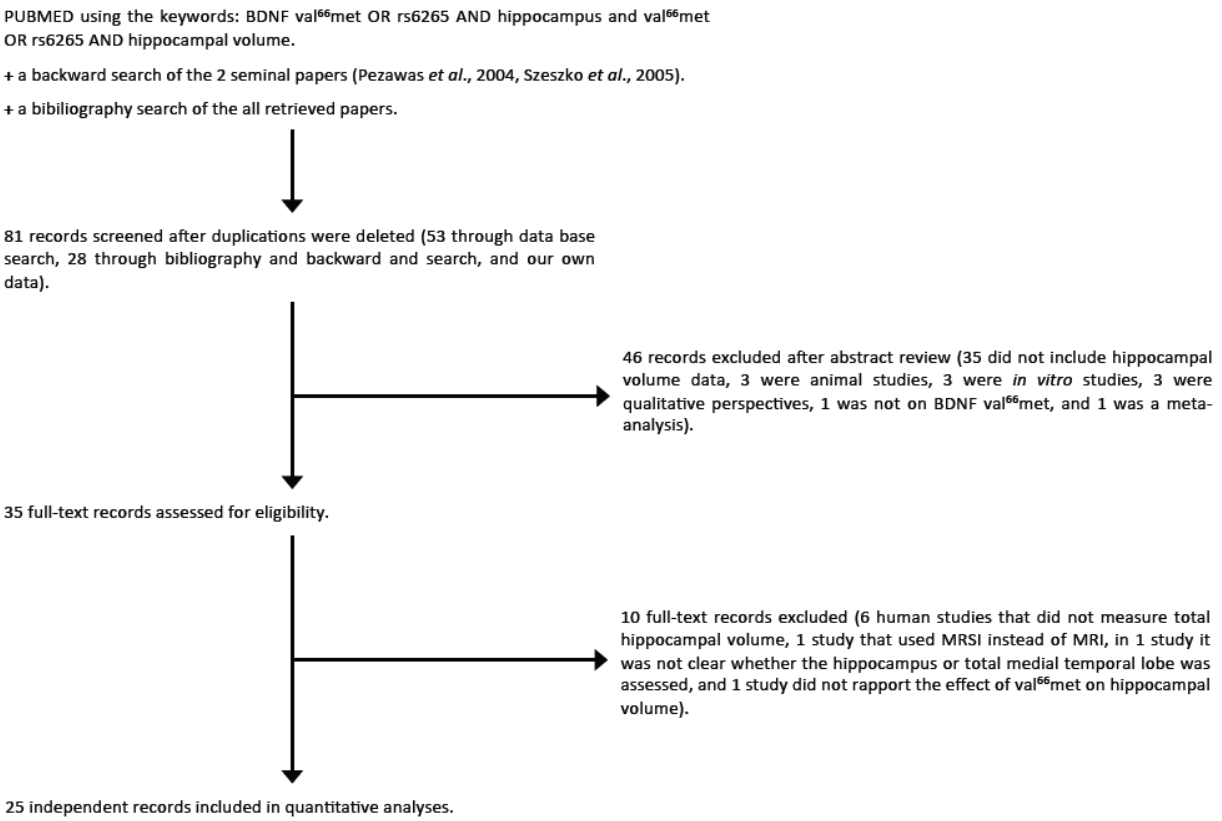

Figure 1. Flow-chart of the search for papers on the association of val^{66}met with hippocampal volume

Data Extraction

We extracted mean total hippocampal volume and Standard Deviation (*SD*) (or *t* and *P* values and the direction of the effect) as a function of BDNF val^{66}met genotype. These outcomes were weighted using inverse variance methods (Borenstein *et al.,* 2009) and converted to standardized Cohen's *d* metrics (Cohen, 1988). Here, a positive value of this metric indicated larger hippocampal volumes in val/val homozygotes relative to carriers of a met allele. In those cases where non-significant results were reported without the necessary statistics to calculate Cohen's *d* (2 records: Karnik *et al.,* 2010; Gerritsen *et al.,* 2011), we assigned the strength of the difference between val/val and carriers of a met allele in hippocampal volume an estimated effect size of 0. Where non-significant results were reported with sufficient information to calculate an effect size, but not the direction of the effect (1 record: Agartz *et al.,* 2006), we assigned the association a Cohen's *d* that was, with regard to its direction, concordant with the study hypothesis. To indicate whether effect size imputation was associated to different effect sizes, we constructed a binary variable indicating whether imputation had taken place. In a meta-regression framework this variable was tested for association with Cohen's *d*. Two of the included records reported longitudinal data (Koolschijn *et al.,* 2010; Millan Sanchez *et al.,* 2012). We included the baseline data of these studies since more subjects were available at baseline compared to follow-up.

In addition to hippocampal volumes and genotype, we extracted data on (**I**) demographical characteristics: mean age, percentage females, ethnicity, Minor Allele Frequency (MAF), and genotype

frequencies of the sample; (**II**) clinical characteristics: psychiatric status (i.e., percentage of the sample with current depression, schizophrenia, or bipolar disorder and the percentage healthy controls of the sample) and psychotrophic medication use (percentage of the sample that used antidepressants, antipsychotics and/or mood stabilizers); and (**III**) methodological characteristics of the study: method of hippocampal volume extraction (VBM versus actual volume measurements), Hardy-Weinberg equilibrium, and whether the hippocampus was traced manually or automatically.

Quality Assessment

We used the criteria set forth by the Strengthening Reporting of Genetic Association Studies (STREGA; Little *et al.,* 2009) and the Strengthening Reporting of Observational Studies in Epidemiology (STROBE; von Elme *et al.,* 2007) checklists using the 11-item list adaption from Karg *et al.* (2011) to evaluate the methodological quality of the included studies. Overall quality score was defined as the frequency of relevant criteria that were met by each individual study. Independent quality assessments were performed by AK and MM. Agreement among the raters proved to be excellent (Cohen's Kappa=0.83, Standard Error (*SE*) = 0.04). Overall, the quality of the included studies was good (mean = 0.86, *SD* = 0.14, range 0.56 - 1.00). Quality ratings of the studies are presented in **Table S1** in **Appendix IV** of this thesis.

Statistical analysis

Meta-analytical calculations were performed using Comprehensive Meta-Analyses version 2.0 (CMA 2.0; Borenstein *et al.,* 2009) with statistical significance set at $P < .05$.

A random effects model was applied to calculate Cohen's *d* (± 95% Confidence Interval (*CI*)) on the difference in total hippocampal volume between val/val homozygotes and carriers of a met allele. Heterogeneity between studies was assessed using the *Q* statistic (Borenstein *et al.,* 2009). Given the possible impact of psychiatric diagnoses on hippocampal volume (MacQueen and Frodl, 2011) meta-analyses and heterogeneity assessments were repeated stratified by psychiatric diagnosis (no diagnosis versus any diagnosis). The difference in effect-sizes that were acquired in these analyses was assessed using a *z* difference statistic.

In a series of meta-regression analyses the possible moderating effects of demographical, clinical and methodological differences across studies on Cohen's *d* were evaluated. The first of these analyses was carried out to test the effects of demographical and methodological characteristics and was run using the data from all included studies. In addition, we tested the clinical characteristic: healthy controls versus any disorder in this analysis. In a second analysis the moderating effects of the demographical and methodological characteristics were assessed using the data from healthy control samples only. This was done to exclude the noise that might have been caused by diagnostic or psychotrophic treatment status of the patient samples. A third analysis was conducted in patient samples to specifically test the moderating effects of psychiatric status (depression versus no depression, schizophrenia versus no schizophrenia, and bipolar disorder versus no bipolar disorder) and psychotrophic medication use (yes versus no). In case of >1 statistically significant moderator, meta-regression analyses were followed up by multivariable regression analysis (SPSS Inc, Chicago, Ill) including the significant moderators in order to learn about their relative contributions to Cohen's *d*.

Publication bias was assessed by funnel plot asymmetry inspection and the Egger test (Egger *et al.,* 1997). In case of publication bias, a trim-and-fill procedure was performed. The trim-and-fill procedure is a procedure that provides an estimation of the effect size after potential bias has been taken into account (Duval and Tweedie, 2000; Peters *et al.,* 2007). Tests of time-related trends were performed by correlating year of publication with weighted Cohen's *d*. Time-related trends were visualized by means of a cumulative

meta-analysis (a meta-analysis that calculates an aggregated effect size for each study that is added to the literature) and scatter-plots. *A posteriori* power and sample size calculations were performed using G*Power (Faul *et al.,* 2009).

RESULTS

Description of samples

The number of subjects of the included studies ranged from *n* = 34 to *n* = 572 (mean = 145, *SD* = 122). In 14 out of the 25 studies (56%) the majority of subjects was female. Mean age of the samples ranged from 23 years to 72 years (mean = 40, *SD* = 14). Eleven of the 25 included studies (44%) reported data on healthy subjects only (*n* = 1,784). The remaining 14 studies (56%) reported data on both healthy subjects (14 subsamples, *n* = 981) and patients with a diagnosis of a psychiatric ilness (depression [7 subsamples, *n* = 431], Schizophrenia or psychosis [6 subsamples, *n* = 345], bipolar disorder [2 subsamples, *n* = 50], and anxiety [1 subsample, *n* = 29]). Some studies (Benjamin *et al.,* 2006 and Gruber *et al.,* 2011) did not provide sufficient information to calculate Cohen's *d* separately for the healthy and the patient samples. Because of this, these studies were not included in the stratified meta-analyses and meta-regression analyses. The numbers in these analyses, therefore, do not add up to the total of *N* = 3,620. **Table 1 ↓** shows basic information on the included records.

Meta-analysis

The results of the meta-analysis over all studies (*k* = 25, *N* = 3,620) showed that carriers of a met allele had lower hippocampal volumes as compared to val/val homozygotes (*d* = 0.13 ± 0.06, 95% *CI* = 0.03 to 0.24, *z* = 2.41, *P* = .02; see **Figure 2 ↓**, panel A for a forest-plot). Analyses stratified by psychiatric diagnosis (no diagnosis versus any diagnosis) revealed similar point estimates for non-patient (*d* = 0.16 ± 0.06, 95% *CI* = 0.04 to 0.27, *z* = 2.57, *P* = .01, *k* = 23, *n* = 2,542) and patient samples (*d* = 0.17 ± 0.11, 95% CI= -0.05 to 0.38, *z* = 1.54, *P* = .12, *k* = 12, *n* = 692)($P_{difference}$ = .96). Substantial heterogeneity across studies was identified in the analyses that were run on the data of all samples (*Q* = 54.47, *P* < .001) on the data of healthy samples (*Q* = 35.11, *P* < .05), and on the data of psychiatric samples (*Q* = 18.11, *P* = .08).

Table 1. Summary of characteristics of studies measuring total hippocampal volume differences between val/val homozygotes and carriers of a met allele at the val[66]met locus presented by year of publication

Author, year	*N*	% female	age	ethnicity	% of *N*	MAF	patient status	% of *N*
Pezawas *et al*., 2004	111	50%	34 [a]	Caucasian	100%	*NK*	healthy controls	100%
Szeszko *et al*., 2005	44	45%	27	Caucasian	100%	0.19	healthy controls schizophrenia	67% 43%
Agartz *et al*., 2006	101	30%	42 [a]	Caucasian	100%	0.19 [a]	healthy controls schizophrenia	51% 49%
Bueller *et al*., 2006	36	61%	27	Caucasian African American Asian	67% 19% 14%	0.21	healthy controls	100%
Frodl *et al*., 2007	120	48%	43	*NK*	*NK*	0.19	healthy controls depression	50% 50%
Miyajima *et al*., 2008	61	68% [a]	63 [a]	Caucasian	100%	0.19 [a]	healthy controls	100%
Takahashi *et al*., 2008	62	40%	25	Asian	100%	0.39	healthy controls schizophrenia	53% 47%
Chepenik *et al*., 2009	34	53%	*NK*	Caucasian African American Other [b]	82% 8% 10%	0.20	healthy controls bipolar disorder	47% 53%
Dutt *et al*., 2009	383	50%	43	Caucasian	100%	*NK*	healthy controls unaffected relatives psychosis	16% 50% 33%
Gatt *et al*., 2009	89	51% [a]	36 [a]	Caucasian	100%	0.20 [a]	healthy controls	
Jessen *et al*., 2009	163	56%	43	Caucasian	100%	*NK*	healthy controls depression	48% 52%
Joffe *et al*., 2009	113	48% [a]	37 [a]	Caucasian	100%	0.21	healthy controls	100%
Schofield *et al*., 2009	161	47%	32	*NK*	*NK*	0.22	healthy controls	100%
Toro *et al*., 2009	331	52%	*NK*	Caucasian	100%	0.20	healthy controls	100%
Benjamin *et al*., 2010	173	65% [a]	69 [a]	Caucasian	100%	*NK*	healthy controls depression	67% 33%
Karnik *et al*., 2010	129	54%	49	Caucasian African American	90% 10%	0.17	healthy controls	100%
Koolschijn *et al*., 2010	177	28%	37	Caucasian	100%	0.20	healthy controls schizophrenia	51% 49%
Cole *et al*., 2011	188	55%	40	Caucasian	100%	0.22	healthy controls depression	59% 41%
Gerritsen *et al*., 2011	572	63%	23	Caucasian	100%	0.23	healthy controls	100%
Gonul *et al*., 2011	73	66%	32	Caucasian	100%	0.25	healthy controls depression	55% 45%
Gruber *et al*., 2011	105	47%	38	Caucasian	100%	0.23	healthy controls schizophrenia bipolar disorder	37% 32% 30%
Kanellopoulos *et al*., 2011	56	63%	72	Caucasian	100%	0.27	healthy controls depression	59% 41%
Richter *et al*., 2011	138	67%	25	Caucasian	100%	0.27	healthy controls	100%
Milan Sanchez *et al*., 2012	43	7% [a]	57 [a]	Caucasian	100%	0.24 [a]	healthy controls	100%
Molendijk *et al*., 2012	157	67%	37	Caucasian	100%	0.18	healthy controls depression [c, d] anxiety [d]	20% 61% 19%

Abbreviations: MAF, Minor Allele Frequency; *NK*, Not Known; e-pub, e-pub ahead of print.
[a] Estimated from larger sample
[b] Not further specified ethnicity, but not Caucasian, African American, or Asian
[c] Included a diagnosis of depressive disorder (*n* = 43, 45%) or comorbid depressive/anxiety disorder (*n* = 52, 55%)
[d] Included a diagnosis of social phobia, panic disorder, generalized anxiety disorder, and/or agoraphobia

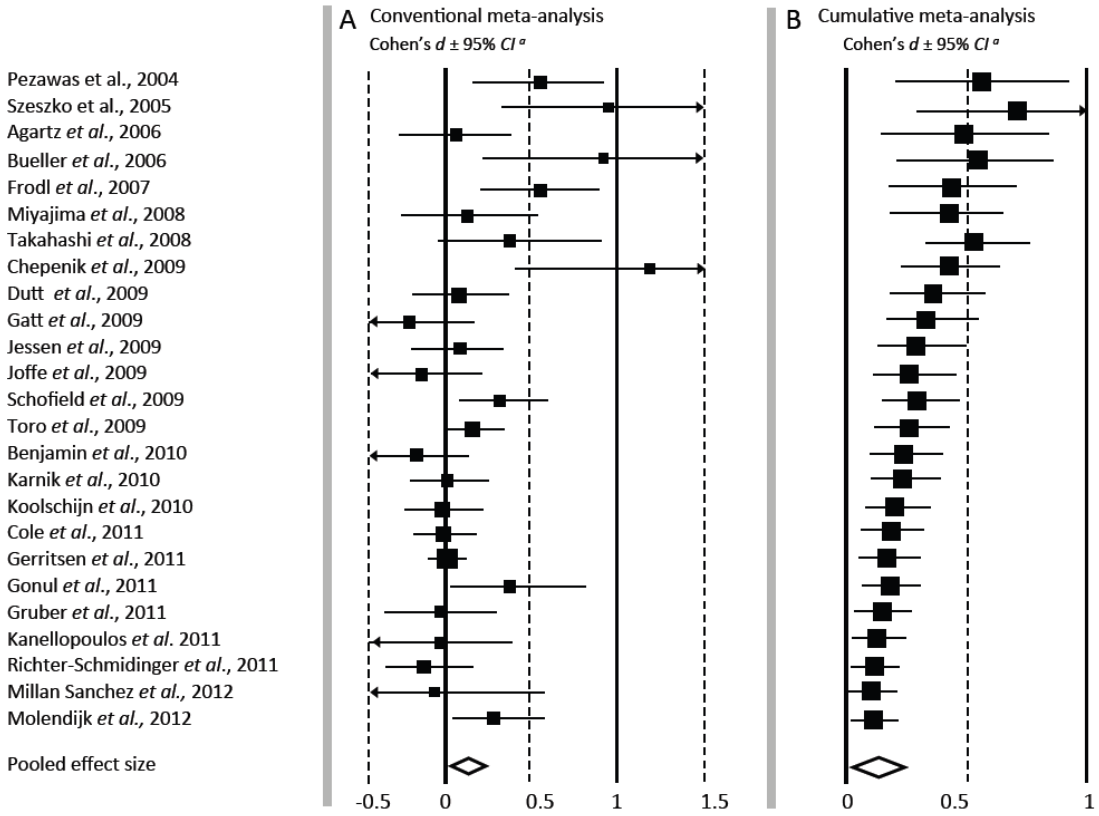

Figure 2. Forrest plot of a conventional meta-analysis (panel **A**, left side of the Figure) and a cumulative meta-analysis (panel **B**, right side of the Figure)

Meta-regression analysis

We evaluated the potential moderating effects of demographical, clinical, and methodological differences across studies in a series of 3 meta-regression analyses. Analyses were conducted separately using the data from all included studies, using the data from healthy control samples only, and using the data from patient samples only. **Table 2** ↓ provides the coefficients that were obtained in these analyses. In sum, mean age of the sample explained a significant amount of variance in weighted *d* (r = -0.43, R^2 = 0.18, P < .05), but most pronounced in the data that were derived from healthy samples. This effect was such that effect sizes were lower in healthy samples in which the subjects were older. Effects of other demographical, clinical, and methodological moderators were not observed. Imputation of effect size (3 records: Agartz *et al.,* 2006; Karnik *et al.,* 2010; Gerritsen *et al.,* 2011) and methodological quality of the included studies also were unrelated to weighted effect size.

Publication bias, and time-related trends, and Sample size calculations

Visual inspection of the funnel plot suggested evidence for publication bias. Egger's test confirmed this (Egger's Intercept = 1.71, 95% *CI* = 0.16 to 3.26, t = 2.29, P = .02). A trim-and-fill estimation suggested that the addition of 2 small and non-significant studies that had to be trimmed and filled would be sufficient to result in a non-significant agregated Cohen's *d* (random effects model) of 0.09 (95% *CI* = -0.02 to 0.22, not statistically significant; see **Figure 3** ↓ for the funnel-plot with observed and imputed values).

Test of time-related trends showed a significant correlation between year of publication (2004 to 2012) and Cohen's *d* (r = -0.54, R^2 = 0.29, P < .01). This effect was consistently found in healthy control samples (r = -0.49, R^2 = 0.24), patient samples (r = -0.55, R^2 = 0.30), and mixed healthy–patient samples (r = -0.55, R^2 = 0.30). The observation that effect sizes decreased over the years is illustrated in **Figure 1**, panel B ↑ (a

cumulative meta-analysis) and in **Figure 4 ↓** (a scatter-plot on the relation between year of publication and effect size).

Table 2. Correlations of demographical, clinical, and methodological study characteristics with Cohen's *d* on the relation between val[66]met and hippocampal volume

	All samples (*k* = 25, *N* = 3,620)	HC samples (*k* = 23, *n* = 2,542) [a]	Patient samples (*k* = 12, *n* = 692) [a]
Demographical/Study characteristics			
Gender (percentage female)	0.04	-0.01	-0.19
Age (mean, years)	-0.36	-0.43* [b]	-0.35
Ethnicity (1 = mixed, 2 = Caucasian)	-0.29	-0.33	-0.40
Minor allele frequency	-0.08	-0.08	-0.14
Sample size	-0.31	-0.31	-0.40
Study quality (frequency of criteria met)	0.12	-0.04	0.45
Clinical characteristic			
Psychiatric diagnosis (1 = no, 2 = yes)	0.18	*NA*	*NA*
Major depressive disorder (1 = no, 2 = yes)	*NA*	*NA*	-0.29
Bipolar disorder (1 = no, 2 = yes)	*NA*	*NA*	0.39
Schizophrenia (1 = no, 2 = yes)	*NA*	*NA*	0.15
Psychotropic drugs (1 = no, 2 = yes)	*NA*	*NA*	-0.22
Methodological characteristics			
VBM (1 = no, 2 = yes)	0.12	0.24	0.01
Magnetic strength (1 = 1.5 Tesla, 2 = 3 Tesla) [c]	-0.09	-0.13	-0.07
Manual hippocampal measurement (1 = no, 2 = yes)	0.31	0.28	0.20

Abbreviations: HC, Healthy Control; *NA*, Not Applicable; VBM, Voxel Based Morphology.
[a] Note. Numbers do not add up to the total *N* of 3,620. This is because some studies (Benjamin *et al.,* 2006 and Gruber *et al.,* 2011) did not provide sufficient information to calculate Cohen's *d* separately for the healthy sample and the patient sample.
[b] Mean age did not remain a statistically significant predictor of Cohen's *d* in a multivariable regression analysis in which year of publication also was added as a predictor variable, whereas the latter did.
[c] One study measured at 1 Tesla [Toro *et al.,* 2009] and was coded as 1.5 Tesla. Excluding this study from analysis did not change the results.
* denotes statistical significance at *P* < .05

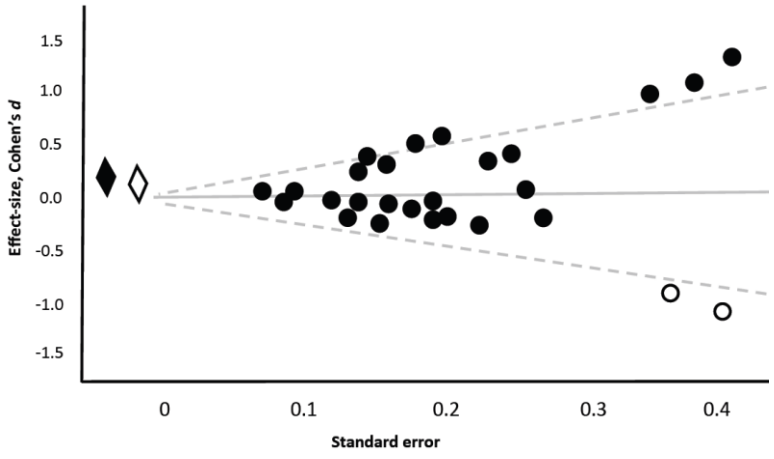

Figure 3. Funnel plot and trim-and-fill estimation showing the typical pattern of publication bias. Filled and open data points depict observed and imputed values respectively. The filed diamond depicts the aggregated point estimate (*d* = 0.13, *P* = .02) and the open diamond the aggregated point estimate after imputation of two studies (*d* = 0.09, not statistically significant).

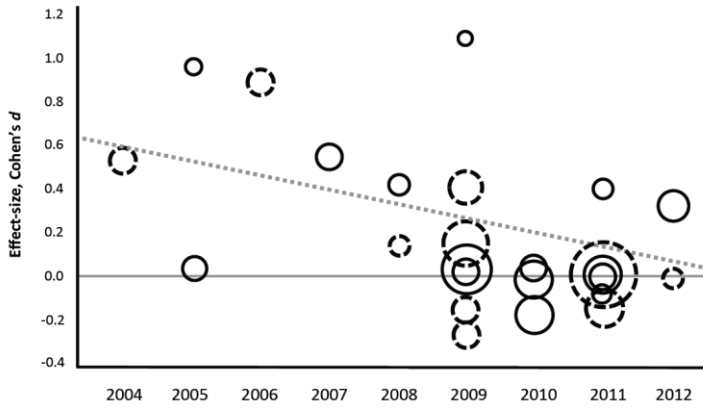

Figure 4. Scatter plot showing the relation between year of publication and standardized Cohen's *d* (weighted by the inverse of the variance) on the association of val^{66}met and total hippocampal volume (Pearson's *r* = -0.54, *P* < .01). Dashed bordered circles indicate studies that included healthy subjects only (*r* = -0.49). Solid bordered circles indicate studies that included both healthy control subjects and patients (*i.e.,* depression, schizophrenia, and bipolar disorder) (*r* = -0.55).

Given that year of publication and age both were significantly associated with effect size these variables were analyzed together in a multivariable regression model. Results of this analysis showed that the effect of year of publication on weighted *d* remained statistically significant (*B* = -0.07, 95% *CI* = -0.14 to -0.01, β = -0.43, *P* = .03) whereas the effect of age disappeared (*B* = -0.01, 95% *CI* = -.02 to 0.01, β = -.35, *P* = .08). It should be noted though that the multivariable statistics should be interpreted with caution when using meta-analysis data because the risk of over-fitting and spurious results (Sterne *et al.,* 2001). Also in our data, if we corrected the standard error for the use of meta-analytic data this relation lost its statistical significance. Notwithstanding this, through data inspection we recognized that the negative association between mean age and Cohen's *d* that we observed in univariable tests, might have been driven by null associations in 2 recently published studies in samples with the relatively high mean ages (~ 70 years; Benjamin *et al.,* 2010; Kanellopoulos *et al.,* 2011). Indeed, if these studies were excluded from the meta-regression, the effect of year of publication remained similar (*r* = -0.47, *P* < .05) whereas the effect of mean age of the sample lost its significance (*r* = -0.29, *P* = .17)

Based on the aggregated effect size we calculated the sample size that is needed to detect a relation between variation at val^{66}met (with the MAF being 0.25) and total hippocampal volume with a power of 0.80 at an α-level of .05. This calculation suggested that 1,900 subjects (1,086 val/val homozygotes and 814 carriers of a met alle) would be neccesary to detect an association of the met allele with total hippocampal volume. Statistical power of the included studies ranged from ~ .07 for the study with the smallest sample size (Chepenik *et al.,* 2009 [*n* = 34] reported effect size *d* = 1.20) to ~ 0.30 for the largest sample size (Gerritsen *et al.,* 2011 [*n* = 572] reported effect size *d* ~ 0). Thus, all the included studies were underpowered.

DISCUSSION

The main goal of this paper was to determine, by meta-analysis, the magnitude and direction of the relation between BDNF val^{66}met and hippocampal volume. Our results, based on 25 samples and a total of 3,620 subjects, suggest that carriers of a met allele have slightly lower total hippocampal volumes (*d* = 0.13) relative to val/val homozygotes. This finding has a plausible biological basis as it can be derived by the findings that BDNF regulates the sprouting and survival of neurons in the hippocampus (Lu and Gottschalk, 2000) and that the met allele is associated with abnormal activity of BDNF in hippocampal neurons (Egan *et al.,* 2003). Hence, the lower hippocampal volume in met carriers is mediated through aberrant trophic support by BDNF. Notwithstanding meta-analytical significance and concordance with biological knowledge, several outcomes of the meta-analyses indicate that the lower hippocampal volume in met carriers is not a genuine biological effect of the met allele but likely has an artificial basis.

Between-study heterogeneity in outcomes in genetic imaging studies may, in general, be due to associations that exist in some populations but not in others or might stem from between-study differences in methodology. Given heterogeneity in a number of characteristics across the studies, it may not be surprising that the reported effect sizes were variable as well (*i.e.,* 7 positive and statistically significant studies and 18 statistically inconclusive positive and negative studies). Through stratified meta-analyses and meta-regression analyses we aimed to identify the sources of this heterogeneity. This is an important venue to pursue as identifying factors that explain variance in outcomes may hint to possible mechanisms that thrive an association. Both types of analyses, however, gave little reason to suspect that heterogeneity in demographical, clinical, and methodological characteristics across studies was systematically related to heterogeneity in effect-sizes. Specifically, we would like to add that manual versus automatic hippocampal volume measurements and the use of 1.5 Tesla versus 3 Tesla also were not associated, structurally, with differences in effect-sizes. An evaluation of the relation between methodological quality of each of the included studies and imputation of effect size similarly showed no relation with Cohen's *d*. This lack of association is an important observation because it justifies the broad set of inclusion criteria that was applied here. However, it should be noted that the use of meta-regression analysis might be hazardous with regard to the occurrence of false positive and negative findings because the number of data-points on which the results of these analyse are based ussualy is rather small (*i.e.,* the number of studies that are included in a meta-analysis; Munafo and Flint, 2004).

Between-study heterogeneity may have artificial sources as well. We detected two such sources in the aggregated data set. First, we consistently observed, over the mixed healthy-patient samples, healthy samples, and patient samples that effect sizes converged closer to null with virtually each subsequent attempt at replication. Second, clear evidence for publication bias was identified. Publication bias typically results from negative studies that are left unpublished and/or from selective outcome reporting (Ioannidis 2011). Together, the decrease in effect size estimates over the years and the publication bias suggest that the observed aggregated effect size (d = 0.13) is an overestimation for the true or most plausible effect size on the association of interest.

Yet another finding from this meta-analysis is that the studies included in our meta-analysis were all underpowered. In fact, *a posteriori* power calculations revealed that the power of the included studies ranged from as low as ~ 0.07 to only ~ 0.30 to detect an effect of the met allele on hippocampal volume. Given that a low level of power increases the ratio of false to true positives (Sterne and Davey Smith, 2001), it seems likely that some false positive findings were among the studies that we included. It should be noted that evidence for increases in the ratio of false to true positives could not directly be extracted from our aggregated data. We did, however, find moderate negative correlation coefficients (albeit non-significant) for the relation between the number of subject in a study and effect size (range: -0.31 to -0.40), that is, larger samples tended to yield smaller effects.

There are some limitations with regard to the methods that were used to detect publication bias. A core problem with regard to the interpretation of funnel-plot asymmetry is that one never knows whether the funnel plot asymmetry is truly due to publication bias or whether it is due to unmeasured differences between studies (Munafo and Flint, 2004). Indeed, heterogeneity in effect-sizes may have come from sources that were not tested in our study, such as the duration or dose of psychotrophic medication use, disease severity, and exposure to stress (MacQueen and Frodl, 2010). Also the key assumption of the trim-and-fill method that the most extreme effect sizes are the ones that are left unpublished has been questioned. However, simulation experiments have shown superiority of the trim-and-fill method above other methods to quantify publication bias when between-study heterogeneity in outcomes is present (Peters *et al.,* 2007).

A limitation that we would like to add is that we could not test the hypothesis of differences in hippocampal volumes between subjects who were homozygous for the met allele (*i.e.,* met/met) and heterozygote val/met subjects, that is a potential dose-dependent effect of the met allele. The frequency of occurrence of the met/met variant is particularly low, at least in Caucasian samples (Petryshen *et al.,* 2011), and none of the included studies reported outcome estimates for this particular variant. Related, the majority of subjects in the studies that were included in our analyses were Caucasian from origin, except for 1 study that reported a positive non-significant effect in an Asian sample (Takahaski *et al.,* 2008). Thus our results might be less applicable for subjects who are of ethnic backgrounds other than Caucasian. In addition, it could be that variation at the val^{66}met locus of the BDNF gene is important for hippocampal morphology only in interaction with childhood trauma exposure for which some evidence exists (Gatt *et al.,* 2009) although 2 of the in this meta-analysis included records (Gerritsen *et al.,* 2011; Molendijk *et al.,* 2012) could not replicate this phenomenon. Another limitation of our study might be that we focused on total hippocampal volume whereas the morphology of the hippocampus is complex and can, for example, be subdivided in a head, a body and a tail (Maller *et al.,* 2007). It could be that the effect of the met allele is limited to morphologically specific sites of the hippocampus (see Montag *et al.,* 2009).

In sum, we carried out a systematic review and meta-analysis on the association between val^{66}met and total hippocampal volume. The results that are reported here indicate that carrying a met allele at the BDNF val^{66}met locus is associated with lower hippocampal volumes. So, one might conclude that the met allele has an effect on hippocampal morphology. However, we observed that effect size estimates converged closer to null with virtually each attempt at replication and that all studies on the subject matter were largely underpowered. Furthermore, we found evidence for publication bias inflating the association reported in the literature. Altogether, this not only suggests that the effect is inherently smaller than reported here (d = 0.13) but also calls into question whether the observed effect is a biological effect of the met allele or whether it is an artifact of underpowered studies. We therefore conclude that variation at the BDNF val^{66}met locus is not likely to account for individual differences in hippocampal volume but rather that the association is subject to a *winners curse*, with large effect sizes found in a few early studies and increasingly smaller effect sizes in later (better-powered) studies.

CHAPTER 10

GENERAL DISCUSSION

The overarching purpose of this thesis was a better appreciation and a more refined model of (peripheral) neurotrophic functioning in depressive (and related) disorders. The empirical data of the prevailing thesis are presented in the previous eight chapters. The next chapter consists of an aggregation and a discussion of these data. This will be done first for the findings on the determinants of serum BDNF concentrations, to be followed by the clinical findings that are reported herein. Strengths and weaknesses will be discussed on the spot and additionally in a paragraph dedicated to this important subject. The discussion will continue with the possible implications of our findings and based on the current state of knowledge the main open questions will be stipulated. A summary in English, Dutch, German, and Spanish closes up this thesis.

Determinants of serum BDNF concentrations

Each empirical chapter in this thesis explored an area that either is directly relevant for our understanding of neurotrophic functioning in psychiatric illness, notably depression, or laid a basic to this end. **Chapters 2** and **3** are examples of the latter as here the determinants of serum BDNF concentrations were explored.

In line with a conceptualization of serum BDNF concentrations as being dependent on a myriad of factors, a long-list of variables was discovered that systematically are associated with serum BDNF concentrations. **Table 1** ↓ summarizes this list next to the main *null findings* that are reported in the **chapters 2** and **3**.

The Basic Determinants of serum BDNF concentrations

Chapter 2 addressed four categories of variables with regard to their association with serum BDNF concentrations. These categories were sampling-, socio-demographic-, lifestyle-, and disease variables.

Of the sampling variables (four were tested; time of blood withdrawal, the number of minutes that a sample was kept in a cool box before being processed, the duration of sample storage, and adherence to the fasting protocol) it was found, in multivariable analyses, that participants who were sampled later in the morning and those who did not adhere to the fasting protocol had lower serum BDNF concentrations. Longer sample storage of blood serum before BDNF determination took place also was associated with lower serum BDNF concentrations. Thus with time, even when stored at -85 C˚, the BDNF protein deteriorates. Herewith we replicate the findings of a Danish group (Trajkovska *et al.,* 2005) that addressed this topic for storage at -20 C˚.

The socio-demographic variables that were tested included gender, age, degree of urbanicity of living environment, and years of formal education. Of these, age was positively associated with serum BDNF concentrations. An age–gender interaction effect further specified this association and indicated that the increase in serum BDNF concentrations related to age was more strongly evident in females as compared to males. This finding was somewhat more complicated as the age related increase in serum BDNF concentrations in women seemed to end when the menopausal stage set in (around 50 years of age; Voorhuis *et al.,* 2011). A possible explanation for this will be provided in a latter part of this discussion. Living in a more urban area was associated with increased serum BDNF concentrations. With regard to the

lifestyle indicators (*i.e.,* smoking, alcohol consumption, the amount of physical activity, and body mass index) it was found that being an excessive drinker was associated with lower-, whereas smoking was associated with higher serum BDNF concentrations.

Table 1. Determinants of serum BDNF concentrations

Category	Variable	Relation to BDNF [1]
Sampling variables (chapter two, *N* = 1,168)	- time of morning blood draw	↓ when sampled later
	- sample in cool box before processing	no association
	- duration of sample storage	↓ when longer stored
	- fasting at times of blood draw	↓ when non-fasting
Socio-demographic variables (chapter two, *N* = 1,168)	- gender	no association
	- age (years)	↑ when higher age
	- level of urbanicity	↑ in urban areas
	- years of education	no association
Lifestyle indicators (chapter two, *N* = 1,168)	- physical activity	no association
	- alcohol consumption	↓ in excessive-drinkers [2]
	- smoking	↑ in smokers
	- body mass index	no association
Disease variables (chapter two, *N* = 1,168)	- metabolic syndrome	no association
	- chronic lung disease	no association
	- coronary artery disease	no association
Meteorological variables (chapter three, *N* = 2,851)	- season of blood sampling	↑ in late summer and early fall
	- month of blood sampling	↑ in September and October
	- ambient sunlight hours	↑ following sunny periods

[1] ↑, ↓: statistically significant higher or lower serum BDNF concentrations respectively
no association: no statistically significant association between serum BDNF concentrations and the variable that is indicated in the corresponding row
[2] excessive-drinking is defined as > 14 units per week

None of the disease related variables, including the presence of chronic non-specific lung- and coronary artery disease, were associated with serum BDNF concentrations. These null findings were not expected based on the literature (see for instance Golden *et al.,* 2010). A possible explanation for this may be the overall health status of the sample and, as a result, a lack of variation in the occurrence of these illnesses and thus low statistical power.

Seasonality in serum BDNF concentrations

Chapter 3 assessed seasonality, a broad concept that refers to biochemical or behavioral alternations as response to variability in the length of day (Walton *et al.,* 2011), in serum BDNF concentrations. A drive to study this topic was that in rodents seasonality has been observed in neuronal plasticity, a process that is regulated by BDNF (Workman *et al*., 2009). A further motivation was that molecular events, upstream of BDNF have been shown to undergo seasonal rhythmicity (Lambert *et al.,* 2002). Finally, depression presumably is related to serum BDNF concentrations (Sen *et al.,* 2008) and this illness occurs to some extent according to a seasonal pattern (Lewy *et al.,* 2006). So, seasonality in serum BDNF concentrations

was expected. And indeed, particular strong evidence for our expectation was found: serum BDNF concentrations increased in the spring-summer period and decreased in the autumn-winter period. This effect was independent of potential confounders such as having a DSM-IV depression diagnosis. Importantly, the observed effect-size estimates for monthly differences in serum BDNF concentrations were substantial (up to a Cohen's *d* of 0.60). Explorative analyses further showed that the number of sunshine hours (a major trigger to entrain seasonality; Walton *et al.,* 2011) in the weeks prior to blood withdrawal positively correlated with serum BDNF concentrations and this partly explained the observed monthly variation. It was also found, and this may not come as a surprise now, that the length of day correlated in a similar manner with serum BDNF concentrations as the number of ambient sunshine hours did.

The findings on the determinants of serum BDNF concentrations have significant implications, as will be discussed in the part that follows.

Determinants of serum BDNF concentrations: implications

Knowledge on the determinants is of importance. Smith and Ebrahim (2002, page 1438) wrote that *we live in an associational world* where people who differ in some regard from others, often differ systematically in other regards as well (*e.g.,* persons who are depressed are more likely to smoke [Kendler *et al.,* 1993]). So, characteristics cluster together and as such they may have shared relationships with certain outcomes. Confounding is said to occur then when one element of a cluster is associated with a given outcome, whereas this relation is due to another element of the cluster. Confounding is the most likely cause of spurious associations (Smith and Ebrahim, 2002) and as such a stand in the way of (research) progress. Gaining detailed insight into determinants or the confounding structure of certain traits, biological alternations or behavior is a means to avoid this because it can provide a scaffold for the exploration of independent associations. The findings in the first two chapters of this thesis provide such a scaffold.

Determinants of serum BDNF concentrations: methodological implications

First, given that the time of the day of blood draw and non-fasting protocol are associated with serum BDNF concentrations, a stringent sample protocol is warranted to obtain valid results. In this protocol a narrow time range should be defined in which blood sampling should take place (*e.g.,* between 07:00 and 07:30 a.m.) and it should be specified and controlled for that participants are sober at the time of blood draw.

Next, since the duration of sample storage impacts on serum BDNF concentrations, when studying differences among diagnostic groups, one needs to make sure that the groups that are compared do not differ in a systematic manner. Let me provide an (oversimplified) example for why this is/becomes (increasingly) important. The US army stores blood from all its soldiers since the start of the first Gulf war in 1991 (*Nature News*, July 2013) to answer relevant questions with. Say, one wishes to learn whether veterans who were sent to Iraq and who did and did not develop post-traumatic stress disorder differ from non-soldier controls with and without PTSD with regard to serum BDNF concentrations. Data on the soldier group comes from the US army database whereas those on the control group need to be gathered after the research question is formulated (let's say June 2014). Given that BDNF levels decrease about 1/10 of a standard deviation each year as a function of storage time (**chapter 2**) the amount of BDNF in the serum of the soldiers (sampled in 1991) would at least be 2 standard deviations lower compared to those of the non-soldier controls (sampled in 2014). Concluding that soldiers who develop post-traumatic stress disorder have lower serum BDNF concentrations as compared to healthy controls with PTSD obviously wouldn't be valid as the between-group difference is due to storage duration.

The discovery of seasonality in serum BDNF concentrations (**chapter 3**) is important for methodological reasons alike. One, it is crucially important when interpreting the results from longitudinal studies. In fact, trials that span months and that have serum BDNF concentrations as an outcome, may be of little use unless detailed knowledge on seasonal effects is taken into account (in the protocol and/or the statistically). Likewise, there is the need to sample groups (*e.g.,* depressed versus healthy controls) equally over the year in order to gain credibility in research findings. Given the seasonal patterns in the occurrence of mood disorders (Lewy *et al.,* 2006) this is quite difficult. I will illustrate this with an example that applies to one of the main findings that is presented in this thesis; the lower serum BDNF concentrations in depressed persons as compared to healthy controls (**chapter 4**, to be discussed in a next part). In the NESDA sample, persons who are depressed are more likely to be sampled in the winter (42 percent of the participants) as compared to the summer (36 percent of the participants). This 6 percent difference may not seem that large but it is statistically significant (*P*-value for the difference = .001) and given the large sample-size of the NESDA a difference of a few percent involves several dozens of persons. Just as the prevalence of depression, serum BDNF concentrations differ as a function of season, with higher concentrations in persons who are sampled in the summer as compared those who are sampled in the winter (d = 0.47, P < .001, see **chapter 3**). Together this already suggests that controlling for season of sampling may make a difference. This indeed is so. Our data shows that the differences in serum BDNF concentrations between depressed patients and healthy control subjects is statistical significant (P = .007) and has an effect size of d = 0.19. When this analysis is rerun controlled for seasonality, the direction of the effect and its statistical significance hold, yet the latter shifts upwards to P = .04 and importantly, the strength of the association is attenuated by about 40 percent to d = 0.11. This is illustrative for the importance of accounting for determinants. Please note that large sample size is more robust with regard to deleterious effects of confounding as compared to studies that conclude on the basis of a smaller sample (Lenth 2001). Therefore the effect-size estimates derived from small-scale studies may be hampered by confounding to a larger extent and thus the results that these yield may not only be less reliable but also less valid.

Concluding from the above, a stringent sample protocol and/or statistical control for a range of relevant variables seem warranted in BDNF related research in order to come to valid results. I gladly noticed that, based on our findings, several authors have picked in their studies on serum BDNF concentrations (*e.g.,* Ball *et al.,* 2013). What the above-presented findings obviously do not bring is insight into how exactly serum BDNF concentrations vary as a function of different levels of *determinant exposure*. this however was not the intention of this thesis and the epidemiological nature of the data that were used gave little room for studying this. Still, some of the findings herein do hint to mechanisms that govern BDNF expression. Given that I consider such hints important in generating future hypotheses, I do not want to simply jump over them. Two illustrative examples therefore are discussed below: (**I**) the interaction effect between age and menopausal stage and serum BDNF concentrations (**chapter 2**) and (**II**) the delayed positive linear relationship between the number of ambient sunlight hours and serum BDNF concentrations (**chapter 3**). Please note that we did not formally test the hypotheses that are brought forward in the following section.

Gender specific associations: estrogen and BDNF

Chapter 2 reports a larger to age related, increase in serum BDNF concentrations in women as compared to men. Given the large number of participants that formed the base for this result (757 women, 408 men), this is not likely to be a chance finding. In addition, we could convincingly exclude that behavioral- or illness characteristics were the source of this association. What could have caused it? Hormonal differences between the two genders seem a viable explanation. In accord with this was that the age related rise in

serum BDNF concentrations in women occurred until the menopausal stage (~ 50 years of age; Voorhuis *et al.,* 2011) and not thereafter. In fact, in female participants it was observed that from ~ 50 years on, age was not associated with serum BDNF concentrations. So, serum BDNF concentrations peak at the climacteric age in women. This is interesting because (**I**) there are studies that have established positive correlations among peripheral estrogen and BDNF concentrations (*e.g.,* Monteleone *et al*., 2007) and (**II**) estrogen expression drops sharply in females in the menopausal stage (Genazzani *et al.,* 1999). Based on this, we hypothesized that the association between age and serum BDNF concentrations could be dependent on menopausal stage and by extension, maybe, on estrogen expression. To probe this further, women in the age range 48 to 52 years were selected and the interaction between age and menopausal status on serum BDNF concentrations was modeled. This analysis showed that in premenopausal women BDNF concentrations increased as a function of age whereas in post-menopausal such a relation was not observed. This corroborates, although not proves, the idea that in women serum BDNF concentrations are under the influence of estrogen expression. This may have clinical implications in that the transition into the menopause is associated with increased odds on depression (Judd *et al.,* 2012) and a relatively large drop in cognitive performance (Farrag *et al.,* 2002), which, according to the neurotrophin hypothesis, are both under the influence of BDNF (Duman *et al.,* 1997). Interestingly, and in line with this idea, is that in **chapter 6** we find that anxious women have lower serum BDNF concentrations as compared to healthy women, whereas in male patients with an anxiety disorder this effect was not observed. This gender specific association could, in theory, also be explained by estrogen because the expression of this hormone is low in anxious women (Walf and Frye, 2006). Some pre-clinical studies suggest the potential importance of this on the level of the central nervous system by showing that estrogen - BDNF interactions are associated with dendritic growth and synaptogenesis in the cerebellum (Haraguchi *et al.,* 2012). So, estrogen and BDNF interactions may be of importance in understanding age and gender related changes in behavior (*e.g.,* depression) and abilities (*e.g.,* cognitive performance). Of course, a manifold of changes occur during the menopausal transition that were not controlled for so alternative explanations loom. Besides, estrogen does not explain some other observations of our studies (*e.g.,* the increase in serum BDNF concentrations as a function of age in males).

Let the sun shine bright: serotonin and BDNF

Our study described in **chapter 3** also yielded some results that hint to mechanisms that govern BDNF expression. Here, I point to the positive relationship between the number of ambient sunlight hours and serum BDNF concentrations. Interestingly, this effect was observed with a delay; where the number of ambient sunlight hours in the weeks prior to blood draw (for up to 7 to 8 weeks before this event) correlated positively with serum BDNF concentrations, the number of sunlight hours in the week of the blood draw itself were not. Importantly, also for this finding we could exclude a range of confounding factors such as the time of the day of blood draw and the study that yielded this finding was well powered.

A possible explanation for this effect can be gained through a comparison with an observation from the treatment setting. Preclinical and clinical studies have shown that antidepressants upregulate the transcriptional activity of BDNF (Duman and Monteggia, 2006) but only after long-term administration (*e.g.,* ≥ 21 days; Conti *et al*., 2002). The gist on why the increase in BDNF expression occurs with a lag time is that antidepressants first increase the availability of monoamines, notably serotonin, to set in CREB activity (Castren *et al.,* 2007). CREB, a general transcription factor (Guilloux *et al*., 2012), binds to the promoter region of the BDNF gene and this positively regulates transcription (Impey *et al*., 2004). Therefore it seems likely that (serotonergic) antidepressants act on CREB through an increase in the availability of serotonin (Impey *et al.,* 2004). Just like treatment with pharmacological antidepressants, long day conditions give rise

to a higher expression of serotonin (Lambert *et al*., 2002). This increase is very likely to occur through a direct effect of light on the expression of L-Tryptophan. L-Tryptophan is known to increase 5-Hydroxy-tryptophan and serotonin expression, leading in turn a higher level of CREB activity and BDNF expression (Castren *et al.,* 2007). Therefore it seems likely that also the delayed increase in serum BDNF concentrations following relatively sunny periods is entrained by increased CREB activation that is induced by a larger availability of biological active serotonin and its precursors L-tryptophan (Cappielo *et al.,* 1996) and 5-hydroxy-tryptophan (Wehr *et al.,* 2001).

An final note on the finding of seasonality in serum BDNF concerns that, although here described as a confounder, it in theory also could be regarded as a mediator linking season and depression. This mediator role could not be excluded in our studies, although it may not seem that likely given the lag-time with which the change in BDNF occurred and the absence of a depression diagnosis times season interaction effect on serum BDNF concentrations.

The determinants of serum BDNF concentrations – recapitulating

In my view, the data that the first two chapters of this thesis bring improve the base to understand inter-individual differences in serum BDNF concentrations. Besides, the acquired knowledge will facilitate ongoing research into neurotrophic functioning in depression (and related illnesses). It allowed us to test, largely independent, some predictions from the neurotrophin hypothesis, which is the topic of the next part of this discussion.

The neurotrophin hypothesis of depression and our work

The rationale for the neurotrophin hypothesis of depression is straightforward: BDNF expression, shaped by genetic and environmental influences, determines neuronal faith and viability and subsequently behavior, including depression (Duman *et al.,* 1997). The two basal predictions from this hypothesis are that depression results from a stress-induced decrease in BDNF expression and that antidepressants are efficacious because they normalize this (Duman and Monteggia, 2006). The overarching purpose of this thesis was to evaluate the validity of these predictions using peripheral BDNF parameters and a genetic variant that is presumed to be associated with neurotrophic functioning. In the section below I will discuss the studies that were performed to this end.

Serum BDNF concentrations in depressive illness

Chapter 4 and **5** advanced the understanding of the associations between serum BDNF concentrations and the illness major depression. **Chapter 4**, a single study, reports in accord with previous findings (Sen *et al*., 2008) and the neurotrophin hypothesis (Duman *et al*., 1997) that serum BDNF concentrations are low in depressed patients as compared to healthy controls (d = 0.19). Importantly, this study also shows that serum BDNF concentrations are low in depressed patients as compared to persons who are in full remission and that serum BDNF concentrations of this latter group are comparable to those of controls. Thus, low serum BDNF concentrations are a state characteristic of depression; an abnormality that is evident during depression and that normalizes during remission.

Chapter 5, a meta-analysis on the same subject, establishes the robustness of this association as it shows, based on 2,384 depressed patients and 2,982 healthy controls that serum BDNF concentrations are low in the depressed state (d = -0.47, 95% *CI* = -0.64 — -0.27). Herewith the neurotrophin hypothesis is corroborated in its prediction that serum BDNF concentrations are abnormally low in depressed patients. What may be the cause of this between-group difference? The axiom that has been brought forward by the neurotrophin hypothesis is trauma- or stress exposure.

Serum BDNF concentrations and trauma/stress exposure

The axiom that for over a decade has been brought forward in explaining depression related alternations in BDNF expression is early life trauma- (such as childhood abuse) or stress exposure in adulthood (Duman *et al.,* 1997; Duman and Monteggia, 2006). Some preclinical and clinical evidence exists for this idea (see for instance Groves 2007). In contrast, the well-powered and well-controlled studies in this thesis show that trauma exposure is not associated with BDNF concentrations (**chapter 7**). In fact, with regard to stress exposure we only could show a negative correlation between recent stress exposure (*e.g.,* a divorce) and serum BDNF concentrations. Given that this association only explained ~ 1 percent of the variance in serum BDNF concentrations it probably does not constitute a sufficient explanation for alternations in neurotrophic functioning in stress related illnesses. The relationship between trauma exposure and BDNF, if it is truly there, probably is more complex and for instance dependent on the presence of a moderator (see **chapter 7** in which a val^{66}met - trauma interaction effect on serum BDNF concentrations was established). This will be discussed in a later part of this discussion. Nothwitstanding a lack of knowledge on moderators, I wish to state that the findings regarding trauma- and stress exposure largely, as they appear to be now (*i.e.,* largely negative) are not in line with the neurotrophin hypothesis. So, maybe other explanations need to be sought for the lower BDNF concentrations in stress related illnesses such as depression. Some findings in this thesis suggest on such mechanisms, other then stress. These putative mechanisms, alongside some some findings that show a lack of fit with the predictions of the neurotrophin hypothesis, will be the topic of the section below.

Serum BDNF concentrations and the early remission phase: reverse causation?

In **chapter 4** we found that patients who were in the early remission phase of their depressive episode, and thus largely free of symptoms, had serum BDNF concentrations that were lower as compared to those of currently depressed patients. Thus, serum BDNF concentrations remain low, or even become somewhat lower, after clinical improvement has set in. Explanations for this could not be elucidated in this thesis and longitudinal designs with frequent samplings need to be performed to understand this issue. Albeit the lack of a clear explanation, the finding of low levels of BDNF in the early remission phase is not in line with the prediction of the neurotrophin hypothesis that low neurotrophic support endangers a person to become depressed (Duman and Monteggia, 2006). In fact, it seems to plead for *reverse causation* in that the lower serum BDNF concentrations do not endanger a person to become depressed but rather are a consequence of being depressed. Why would serum BDNF concentrations be particularly low in the early remission phase of depression? Two hypotheses will be formulated here that potentially can explain it: (**I**) to depression related changes in body-weight and (**II**) to depression related changes in levels of oxidative stress. These hypotheses will be discussed below.

In **chapter 4** we find that BMI is positively associated with serum BDNF concentrations in depressed patients (note that such a correlation was not observed in healthy control subjects, see **chapter 2**). Although this finding was unsought, it parallels the results of some previous studies (Nakazato *et al*., 2003; Monteleone *et al*., 2005) and they give ground to an interesting hypothesis. As weight loss is a prime behavioral abnormality of depression (APA 1994) and often a residual symptom in early remission (Paykel 1985; Paykel *et al*., 1995) it could be that alternations in serum BDNF concentrations are mediated by (transient) changes in eating behavior during, or in the aftermath of a depressive episode. The mechanism would then simply be that a decrease in intake of the building blocks for the protein BDNF could lower the expression of it or in this case particular, a higher metabolism of BDNF and consequently lower BDNF concentrations. Likewise, weight gain is a documented side effect of antidepressant treatment (Kachur *et*

al., 2005). And this, or better the absence of weight loss could potentially explain the absence of a relative fall of serum BDNF concentrations in depressed patients who are treated with an antidepressant (the associations among antidepressants and serum BDNF concentrations will be discussed in a latter part of this discussion).

Another explanation for the abnormally low serum BDNF concentrations in the early remission- and the active depression phase is mediation by oxidative stress. Depressive disorders are accompanied by a decreased antioxidant status (Maes *et al.,* 2011). The antioxidant status of a person refers to the capacity to protect against reactive oxygen species. An imbalance of the oxidative status generates toxic reactive oxygen species and this causes damage to membrane lipids, to DNA and consequently disturbs the functioning and stability of proteins (Sarandol *et al.,* 2007). So, oxidative stress causes oxidative imbalance with accompanying protein damage and also BDNF functioning may be negatively affected by it. Alternations in oxidative stress homeostasis set in during the depressed state (probably due to behavioral alternations such as a changed eating pattern and less physical activity). Therefore also the lowering of BDNF functionning, if it truly is affected by oxidative stress, may only set in during the depressed state (critically, not prior to the depressed state as the neurotrophin hypothesis suggests). Given that the disturbed oxidative stress homeostasis may linger on into early remission (Barnham *et al.,* 2004), BDNF levels consequently may remain low in this phase. There is one study in human subjects that confirmed the idea on the role of oxidative stress on neurotrophic functioning to some extent. This study by Kapczinski *et al.* (2008) showed a negative correlation (r = -0.58) between serum thiobarbituric acid reactive substances and BDNF concentrations in bipolar patients (in whom serum BDNF concentrations in general are low; Fernandes *et al.,* 2011). This is suggestive for the notion that alterations in oxidative stress homeostasis may be mechanistically associated with alternations in the expression/metabolism of BDNF. There are some preclinical studies that support this idea. Already in 1996, Kirschner and her colleageaus showed, *in vivo*, that neuronal damage and decreased BDNF expression can be induced by chemical hypoxia. This, however, could be attenuated by BDNF administration. Interestingly, some authors have suggested that antidepressants may affect oxidative stress homeostasis in a positive manner (Khanzode *et al.,* 2003). Therefore, normalization of oxidative status may complementary explain the absence of abnormally low serum BDNF concentrations in antidepressant treated depressed persons. Finally, is notable that several studies show that oxidative stress is associated with processes that typically are governed by BDNF, such as neuronal functioning (Barnham *et al.,* 2004).

Whatever the mechanism that is involved in the lowering of serum BDNF concentrations in the depressed and the early remission phase, **chapter 4** suggests that the effect-size on these differences are small in absolute sense (*i.e.,* a standardized mean difference of ~ 0.2) and also when compared to those reported by earlier small scale studies and meta-analyses (see for instance Sen *et al.,* 2008). Small effect-sizes indeed were confirmed in the meta-analysis, *the way to converge to the true effect-size*, in **chapter 5**. The findings of this chapter and the implications that they may have are discussed below.

Serum BDNF concentrations and depression diagnosis – small effect-size estimates

Chapter 5, a large-scale meta-analysis, shows lower serum BDNF concentrations in untreated depressed patients as compared healthy controls. This finding is not new (see above). The novelty of the work instead is that it highlights a large amount of between-study heterogeneity in outcomes. Importantly, none of the theoretically relevant variables that we tested (*e.g.,* gender distribution of the sample) was associated with the between-study heterogeneity. Obviously, it may have come from between-sample characteristics, such as heterogeneity in clinical characteristics of patient samples. However, for this idea, meta-regression analyses could find no evidence whatsoever. In contrast, these analyses showed an artificial base for the

heterogeneity in outcomes. First, a large part of the studies that were included was underpowered. Given that a low level of power increases the false versus true positive ratio (Sterne and Smith, 2001), some overly positive findings may have been among the studies that we included, causing heterogeneity. Second, we found that sample size and year of publication were significant predictors of between-study heterogeneity, with larger and more recently published studies reporting smaller between-group differences. This points to publication bias – which is a threat to the validity of the literature and besides a cause of hererogeneity. Analyses that accounted for publication bias yielded an attenuated effect-size estimate that was about half as large (*i.e., d* = 0.47) as the one reported in previous meta-analysis that was based on 8 times less data (Sen *et al.,* 2008). Of course, small-effect sizes do not attest the validity of a hypothesis. In fact, the effect-size estimate remained statistically significant and thus corroborates it. What this finding does attest however is that the data, for reasons of publication bias and overestimations of effect-sizes, should be critically interpreted. A consequence of this is that the evidence for the neurotrophin hypothesis is slimmer as was initially thought. This is in line with more recent work showing that, using longitudinal designs, serum BDNF concentrations are not, or only marginally, predictive for depression related psychopathology (*e.g.,* Vinberg *et al.,* 2013; Bus *et al.,* submitted). Furthermore, the findings in this thesis on the lack of an association between serum BDNF concentrations and the clinical features of depression and all major types of antidepressants use (**chapters 4** and **5**) also suggest that the evidence for the neurotrophin hypothesis is not so strong as was initially thought. This will be discussed in below.

Serum BDNF concentrations and the clinical features of depression

The putative association between serum BDNF concentrations and the symptom severity of depression has been brought forward as a pillar of the neurotrophin hypothesis (*e.g.,* Karege et al., 2002). This makes sense because when you predict that serum BDNF concentrations play a role in the pathophysiology of depression as the neurotrophin hypothesis does (Duman and Monteggia, 2006) you may expect that BDNF levels are are particularly low in more severely depressed patients. The findings reported in **chapter 4** and **5** however are not in line with this expectation. The single study reported in **chapter 4** could not replicate the association between depression symptom severity and serum BDNF concentrations in unmedicated depressed persons (r = 0.03, P = 0.23, N = 541) neither could the meta-analysis in **chapter 5**. The meta-analysis added a layer of certainty to this, as it showed that the studies that did find evidence for this association are outliers and likely false positives. So, the dose-response association between serum BDNF concentrations and the symptom severity of depression probably does not exist. This further is exemplified by the finding in **chapter 4** that the early remission phase is accompanied by a much lower symptom severity of depression (mean depressive symptom severity scores were 22.4 ± 11.4 versus 32.4 ± 12.1 (P < .000001) in currently depressed patients) alongside lower BDNF concentrations as compared to the current depressed state.

Regarding the other clinical characteristics of depression, **chapter 4** also could not confirm the findings that having a recurrent compared with a first episode of depression (Lee *et al*., 2007) and the presence of suicide ideation (Deveci *et al*., 2007; Kim *et al*., 2007) are accompanied by lower concentrations of BDNF. Age at onset of depression, the presence of comorbid anxiety and the chronicity of depression also were shown to be unrelated to serum BDNF concentrations (see **chapter 4**). Because most of the studies in the literature did not report on these variables there was no opportunity to confirm the null-findings regarding these clinical characteristics through meta-analysis. Notwithstanding this, together these findings, given the samples-sizes that were used to come to them, give confidence in excluding the clinical features of depression as potential correlates of serum BDNF concentrations. This is an important conclusion, as it

hints that other factors than specifically to depression related phenomenon, such as weight gain and loss, may be at play in the relative fall of BDNF concentrations during/around a depressive episode.

Serum BDNF concentrations and antidepressant treatment

A core prediction of the neurotrophin hypothesis is that antidepressants are clinically efficacious because they normalize neurotrophic functioning and with this aberrant brain functioning (Duman and Monteggia, 2006). In the **chapters 4** and **5** we find some evidence for this prediction.

Within the context of a large-scale single study (**chapter 4**) we found evidence that serum BDNF concentrations normalize in the course of antidepressant treatment. This finding largely is in accord with previous findings (Brunoni *et al*., 2008). It should be noted though that the effect-size on this association is considerably smaller (*i.e., d* = 0.23) as compared to the effect-sizes that previous studies report on. Still, in **chapter 5** the reliability of this finding was confirmed through meta-analysis, albeit this analysis also yielded a considerably smaller effect-size estimate (*i.e., d* = 0.34) as compared to those in previous studies (*i.e., d* = 0.80; Sen *et al.,* 2008). Besides, we were able to expand previous findings by showing that the use of an antidepressant is associated with increased serum BDNF during a depressive episode but not during remission. This suggests that antidepressant-induced increases in BDNF occur in a disease state when BDNF functioning might be defective and not in full remission when BDNF functioning is normalized (see **chapter 4**).

Interesting was that the increase in serum BDNF concentrations appeared to be specifically associated with the use of SSRIs and St John's wort and not with the use of SNRIs, TCAs or NaSSAs (**chapter 4**). Although not directly confirmed, this finding might be explained by increased availability of extra-synaptic concentrations of serotonin, as it is known that serotonin stimulates the expression of BDNF (Martinowich and Lu, 2008). In line with this, we found the highest BDNF concentrations in patients who were treated with agents that generally lead to an increase in the availability of serotonin; SSRIs and St John's wort (Gaster and Holroyd, 2000). Furthermore, the lowest concentrations of BDNF were found in patients who were treated with NaSSAs that are known to have little or no impact on the availability of serotonin (Antilla and Leinonen, 2001). Noteworthy is that this observation, and its putative explanation in terms of the availability of serotonin fits very well with the findings on seasonality in serum BDNF concentratons (see **chapter 3**). The antidepressant specific effect on serum BDNF concentrations however could not be replicated in the meta-analysis reported in **chapter 5**. This could be due to a lack of statistical power as in this analysis it had to be assessed through a meta-regression that used the number of included study as data-points and this number was only 28.

Notwithstanding the findings that seem to confirm the neurotrophin hypothesis, the results that were described in **chapter 4** and **5** do not all recapitulate it. First, the antidepressant-specific effect on serum BDNF concentrations (already mentioned above) seems at odds with the specific prediction of the neurotrophin hypothesis that increases in BDNF concentrations are a key mediator for an antidepressant response to occur (Duman and Monteggia, 2006). According to this prediction, one might expect that antidepressants that are known to be about equally efficacious in the treatment of the symptoms of depression (Berton and Nestler, 2006) would have similar effects on serum BDNF concentrations. Clearly, this is not the case.

A second finding that seems hard to reconcile with the neurotrophin hypothesis is that the group of depressed persons who used antidepressants (for prolonged period and on a frequent base) had the highest BDNF concentrations, but also the highest symptom severity of depression (**chapter 4**). This suggests that increases in peripheral BDNF concentrations do not parallel clinical effectiveness, or at least have no direct effects on depression characteristics such as its severity. Thus, the temporal dynamics as

predicted from the neurotrophin hypothesis do not seem to be correct. Furthermore, epiphenomena for the finding that antidepressants are associated with serum BDNF concentrations cannot be excluded. Interestingly, among these epiphenoma are those that also could explain differences among untreated depressed persons and healthy control subjects (*i.e.,* to depression and treatment related weight gain and loss and oxidative stress levels). Finally, **chapter 5** reports, along a similar line as the findings regarding differences among diagnostic groups, that a large amount of unexplained between-study heterogeneity in outcomes and publication bias is evident in the literature on the association between antidepressants and serum BDNF concentrations. This also indicates that the effect of antidepressants on serum BDNF concentrations is not that large as they initially were thought to be.

Relevant to the above-presented discussion on lower BDNF concentrations in persons diagnosed with depression are the findings reported in **chapter 6**. In this chapter serum BDNF concentrations were evaluated in persons diagnosed with an anxiety disorder. Given that the anxiety disorders mimic depressions to a great extent (David et al., 2009), abnormalities in serum BDNF concentrations were expected. This putative association is the topic of the section that follows.

Serum BDNF concentrations and anxiety

Based on animal models (*e.g.,* Monteggia *et al*., 2007), some small-scale human studies (Strohle *et al*., 2010), and the neurotrophin hypothesis (Duman and Monteggia, 2006) there is a strong a priori reason to expect that serum BDNF concentrations are low in persons who suffer an anxiety disorder. However, robust evidence for this belief is absent. **Chapter 6** filled this gap and explicitely tested the hypothesis that serum BDNF concentrations are low in patients with an anxiety disorder as compared to healthy controls. The results of this study, controlled for a range of demographical and behavioral variables and derived from a sufficiently powered design however did not confirm this hypothesis as overall no differences in serum BDNF concentrations between patients with an anxiety disorder and healthy controls were found. So, it seems unlikely that BDNF is involved in the pathophysiology of anxiety disorders *per se*.

Given that the preclinical literature gives ground to test for gender differences in the relation between anxiety and BDNF (Govindarajan *et al*., 2006), we assessed gender differences as well. Analyses stratified by gender revealed that female patients had somewhat lower BDNF concentrations relative to female controls (d = 0.19), whereas BDNF concentrations were similar among male patients and male controls. This gender specific finding could point in the direction that BDNF is related to the pathophysiology of anxiety in female but not in male patients. Remarkable is that this finding compares well with some studies from the depression literature, which have shown lower concentrations of BDNF in female depressed patients as compared to male depressed patients (Karege *et al*., 2002a; Huang *et al*., 2008; and **chapter 4**).

The origins of this gender specific finding are unknown. Here, and also in depressed subjects (see **chapter 4**) we found that the differences in serum BDNF concentrations between female and male patients were not driven by demographical (*e.g.,* age), behavioral (*e.g.,* smoking), or clinical (*e.g.,* severity) variables. In **chapter 6** it further was shown that the difference between female and male patients could not be attributed to a specific subtype of anxiety. A general deduction from this, and from the finding that serum BDNF concentrations are similar among female and male controls, is that the origins of it may lie in a female specific associate of anxiety. One interesting candidate that might serve as an explanation is the expression of the ovarian hormone estrogen, which in women with an anxiety disorde typically is low (Seeman, 1997; Almeida *et al*., 2005; Walf and Frye, 2006). This is of relevance here because estrogen is a signaling molecule that triggers the expression of BDNF (Scharfman and MacLusky, 2004; Begliuomini *et al*., 2007). This explanation relates to the observation in **chapter 2** that in women serum BDNF concentrations seem to peak at the climateric age (which already is discussed in an earlier part of this discussion).

Alternatively, and also not unlikely, the lower BDNF concentrations in female patients may be a female specific artifact of being anxious that is (causally) unrelated to the disease state itself. Therefore, and because of the findings that males did not show anxiety related lower serum BDNF concentrations, I conclude that it is unlikely that BDNF is involved in the pathophysiology of anxiety disorders *per se*. This conclusion is in contrast to the neurotrophin hypothesis (Duman and Monteggia, 2006) that patients with to depression related conditions exhibit abnormally low neurotrophic support. A final finding from **chapter 6** that deserves to be noted is that a history of depression seems unrelated to serum BDNF concentrations in patients with a current anxiety disorder, which corroborates our finding that low serum BDNF concentrations are a state characteristic of depression (see **chapter 4**).

The gender specific association and the low effect-size estimates that are reported in this thesis bring me to the topic of clinical utility. Studies into neurobiological abnormalities in psychiatric illness, BDNF in the prevailing thesis, may serve two functions: (**I**) they may help to parse out the pathophysiology of a certain illness condition and (**II**) they may add in the classification of a diagnostic condition or in the prediction of how successful a given treatment will be. The second function*, clinical utility*, will be discussed in some detail in the section that follows.

Clinical utitlity: diagnostic and treatment biomarkers

I will start this section with a short introduction on the concepts biomarker, moderation and mediation.

As stipulated in an earlier part, depressive disorders nowadays are diagnosed based on subjective assessments. This comes with disadvantages, as they may be inaccurate and/or colored by the state a patient is in. Therefore it is generally believed that an objective biological marker could improve the diagnostic process (*i.e.,* a *diagnostic biomarker*; Schmidt *et al.,* 2011). In addition, a biomarker could help to reduce heterogeneity by classification in a finite number of illness subtypes, which could have as advantage that patients can be assigned to treatment options that best fit their needs (Schmidt *et al.,* 2011). This latter distinction can be regarded as a treatment *moderator*; a differentiation that is used to decide for whom a certain treatment will work: depression type A → antidepressant X → depression alleviation *versus* depression type B → antidepressant Y → depression alleviation. Several biological markers have been studied in relation to depression (*e.g.,* cytokines, metabolic markers; see Kapur *et al.,* 2012), yet so far without success in that none of them have led to true clinical gain. A well-known example of this is the dexamethasone suppression test, which in the 1970/80s had initial promise in predicting relapse into depression. After extensive and sufficiently powered studies, this test however appeared to have a rather low sensitivity (~ 40-50%) and specificity (~ 70%)(APA taskforce on laboratory tests in psychiatry, 1987) and therefore could not be translated in clinical utility.

The concept of treatment mediator can be described along a similar line. A mediator however is different from a moderator in that it describes a mechanism by which a treatment may work (*i.e.,* a treatment mediator: depression → treatment → increase in biomarker X → depression alleviation). Information on treatment mediators also would be of clinical help in that objective assessment early in the course of treatment may be used as a marker for (early or future) treatment success and hence may improve drug efficacy (Schmidt *et al.,* 2011) and the understanding of the mechanisms that underlie antidepressant action.

BDNF as biomarker

Could serum BDNF concentrations possibly serve a biomarker function? Around the time that I started my thesis (2008) the answer on this question was *yes*. Sen *et al.* (page 527) for instance, concluded their 2008 meta-analyses on 748 subjects of whom 366 were depressed that serum BDNF concentrations *may have*

use as a biomarker for major depressive disorder or antidepressant efficacy. The effect-sizes on which this conclusion was based (*i.e.,* Cohen's *d* ~ 0.80) made their conclusion seemingly valid. However, and looking fairly at an effect size of 0.80, what does it tell us and can it be applied to distinguish between-groups in a diagnostic setting? Yes, it can, but only to a certain extent (i*.e.,* probability that an individual is correctly classified based on it (only) is 0.66 [Coe 2002]).

We report in **chapter 4** and **5** effects sizes on between-group differences that are considerably smaller (*e.g.,* for the difference between untreated depressed persons and healthy controls the best estimate is *d* = -0.47 (95% *CI* = -0.64 — -0.27). How well can a person be assigned to a group based on such an estimate? Not so well. In fact, the probability of correct classification is .59. Please note that an effect size of 0.00 would yield a .50 correct classification. Given that the relevance of a diagnostic biomarker depends on the magnitude of an effect-size (and not on statistical significance), I conclude that serum BDNF concentrations are lof little, if any, clinical use.

What is more is that lower BDNF concentrations have been reported in persons diagnosed with schizophrenia, with bipolar disorder, with eating disorders, etcetera. In **Table 2 ↓** (next page) the most reliable evidence (*i.e.,* derived from the largest single study or from meta-analysis) for alternations in serum BDNF concentrations in some psychiatric and neurological illnesses is presented. From this table it becomes clear that low serum BDNF concentrations are not specific enough to differentiate among diagnoses. So arguably, these values are very little informative in the clinical setting. Another reason why the issue of BDNF as a diagnostic biomarker may need a nuanced approach is that basically all the findings are acquired from between-subjects designs and therefore the data apply to groups and not to individuals. Furthermore, for the clinical use of BDNF parameters the detailed knowledge on the myriad of factors that influence BDNF concentrations also should be taken into account, making the assessment of BDNF concentrations pretty complex.

Although limited in scope with regard to clinical utility, our findings on between-group differences do not necessarily dismiss the possibility that abnormalities in BDNF expression reflect a pathophysiological process that may underlie depression (Duman *et al.,* 1997). Even more, the magnitude of the difference in serum BDNF concentrations between depressed patients and healthy control subjects that we report on (*e.g., d* = -0.47 for depressed persons versus healthy controls) stands out as strong when compared to other biological abnormalities in depression, for instance blood markers for immune dysregulation (*e.g.,* CRP [*d* = 0.15]) or HPA-axis activity (*e.g.,* adrenocorticotropin hormone [*d* = 0.28] see Penninx *et al.,* 2013). Furthermore, in the meta-analysis reported in **chapter 5** we find that a greater increase in serum BDNF concentrations in the course of antidepressant treatment is associated with a larger decrease in depression symptom severity. This finding may fuel work into the theoretically and clinically relevant topic on the temporal dynamics between BDNF expression and treatment efficacy. It would be interesting if future studies could address early changes in the course of (pharmacological) treatment, a notion for which some evidence exists (Lang *et al.,* 2006; Machado-Vieira *et al.,* 2009) and on which Maryna Polyakova (Max Planck Institute Leipzig, Germany) and I are writing a systematic review (work in progress).

Table 2. Alternations in serum BDNF concentrations in other psychiatric/neurological illnesses than depression (in alphabetical order). Magnitude of the difference is expressed as standardized Cohen's *d* versus a healthy control group

Disorder, author, year	Design	Finding
ADHD Corominas-Roso *et al*. (2013)	single study	BDNF concentrations are low in patients with ADHD (*n* = 54)[*d* = -0.80]
Alcohol dependency Huang *et al.* (2011)	single study	BDNF concentrations are low in alcohol dependent patients (*n* = 65)[*d* = -1.24]
Alzheimer's disease Yatsutake *et al*. (2006)	single study	BDNF concentrations are low in patients with Alzheimer's disease (*n* = 60) [*d* = -0.77]
Autism Hashimoto *et al*. (2006)	single study	BDNF concentrations are low in patients with autism (*n* = 18)[*d* = -1.58]
Bipolar Disorder Fernandes *et al.* (2011)	meta-analysis	BDNF concentrations are low in patients with bipolar disorder (*n* = 548) in the manic- and depressed state [[*d* = -0.8, 95% *CI* = -1.1 — -0.5] and *d* = -0.94, 95% *CI* = -1.72 — -0.53 respectively]
Eating disorders Monteleone *et al*. (2011)	single study	BDNF concentrations are low in patients with anorexia nervosa (*n* = 27) and bulimia (*n* = 24)[*d* = -1.54 and -1.26 respectively]
Huntington's disease Ciammola *et al*. (2007)	single study	BDNF concentrations are low in patients with Huntington's disease (*n* = 42)[*d* = -1.71]
Schizophrenia Green *et al*. (2010)	meta-analysis	BDNF concentrations are low in schizophrenic patients (*n* = 1,114)[*d* = -0.53, 95% *CI* = -0.81 — -0.18].

Abbreviations: ADHD, Attention-Hyperactivity Disorder; BDNF, Brain-Derived Neurotrophic Factor

Noteworthy in this context, and of high importance for future progress, is a recent argument by Steve Hyman (Broad Institute, Cambridge) who said in *Nature news* (May 10, 2013) that *It's a fool's errand to try to find a biomarker for a diagnosis with little basis in nature … such efforts waste human capital and governmental and industry funds.* This makes a lot of sense: the broad nosological categories that in general are used in psychiatric research pose serious limitations in the possibilities to detect (biological) abnormalities (Casey et al., 2013) because they are not valid. Searching for associations beyond the boundaries of diagnostic categories therefore may be worth considering as an important innovation. It could for instance be considered whether single, or less broad, and more carefully defined domains that may constitute the illness depression (*e.g.,* motivation or reward) in particular are correlated with BDNF. With my colleague Boudewijn Bus (Radboud University Nijmegen, The Netherlands) and others I have tried to make some advance in this (Bus *et al.,* 2013). This enterprise however yielded little additional insight. So, and given that BDNF alternations are observed in many psychopathological conditions (see **Table 2 ↑**), studies could relate alternations in BDNF to trans-diagnostic phenomena (*e.g.,* rumination; Beevers *et al.,* 2009; oxidative stress; Kapczinski *et al.,* 2008, sleep; Giese *et al.,* 2013, weight gain and loss; Monteleone *et al.,* 2007), or the research domain criteria (Casey *et al.,* 2013).

Two know more than one

Given that the above feed my concerns on the relevance of serum BDNF concentrations with regard to depression (either as a biomarker or a factor contributing to its pathophysiology) and debate on these issues in the literature I decided to ask the opinion of colleagues on this issue. Heretoo, I ran a poll in which I asked 100 researchers (who were corresponding author for published papers that had *BDNF* and *depression* (*n* = 50) or *cognitive/interpersonal* and *depression* (*n* = 50) [the latter group was included to reduce bias] in their title) about this. The results of this poll are described in detail in **Appendix V**. The majority of researchers that responded (*n* = 60) either agreed (43 percent) with the proposition that serum BDNF concentrations are relevant with regard to depression *or* expressed the belief that the future will

inform us on this issue (42 percent). Only 15 percent explicitly disagrees with the notion that serum BDNF concentrations relevant with regard to depression. In this sense, the poll was helpful in that most authors see either relevance in the use of serum BDNF concentrations as parameters for depression or suggests that more research will bring definite answers.

Recapitulating our work on serum BDNF concentrations in relation to the neurotrophin hypothesis

Table 3 ↓ gives an overview of the findings on serum BDNF concentrations and how these relate to the predictions from the neurotrophin hypothesis. Conclusions will follow in a later part of this discussion.

Table 3. Summary of the research findings in this thesis on serum BDNF concentrations and how they fit with the neurotrophin hypothesis (confrimative *versus* non-confirmative)

Confirmative
1. Serum BDNF concentrations are low in depressed patients relative to healthy controls (**chapter 4** [*N* = 923] and **chapter 5** [*N* = 5,203]).
2. Serum BDNF concentrations are normalized in the course of depression remission (**chapter 4** [*N* = 1,080] and **chapter 5** [*N* = 4,204]).
3. Serum BDNF concentrations are normalized in the course of antidepressant treatment (**chapter 4** [*N* = 1,080]; **chapter 5** [*N* = 4,204]).
4. A larger increase in serum BDNF concentrations is associated with a larger decrease in depressive symptoms over the course of antidepressant treatment (**chapter 5** [*N* = 1,422])
5. Serum BDNF concentrations are low in female patients with an anxiety disorder relative to female controls (**chapter 6** [*N* = 499]).
6. Exposure to recent stressful events is associated with lower serum BDNF concentrations (**chapter 7** [*N* = 1,435]).
Non-confirmative
1. Serum BDNF concentrations are low in the early remission phase of depression as compared to the depression state (**chapter 4** [*N* = 541])
2. Serum BDNF concentrations are normalized in the course of treatment with an antidepressant but this is not associated with remission (**chapter 4** [*N* = 421]).
3. Equally effective pharmacological antidepressants are differently associated with serum BDNF concentrations (**chapter 4** [*N* = 421]) [A]
4. Clinical characteristics (notably depression severity) are not associated with serum BDNF concentrations (**chapter 4** [*N* = 541] and **chapter 5** [*N* = 9,484]).
5. The differences in serum BDNF concentrations as a function of diagnostic and treatment status are overestimated and are of a small effect-size at best (**chapter 5** [*N* = 9,484]).
6. Serum BDNF concentrations are normal in male patients with an anxiety disorder relative to male controls (**chapter 6** [*N* = 276]).
7. Childhood trauma exposure is not associated with serum BDNF concentrations (**chapter 7** [*N* = 1,435]).

[A] Note that the possibility exist that different types of antidepressants may be clinically efficacious through different mechanisms (Mann 2005) and that therefore this finding is not neccesarly non-corfirmative with te neurotrophin hypothesis.

Besides our studies on serum BDNF this thesis explored associations between variation on the gene that codes for BDNF (val^{66}met) and depression-related phenotypes. These explorations are discussed in the following section.

BDNF val^{66}met and the neurotrophin hypothesis

The genetic studies in this thesis focused on one particular polymorphism on the BDNF gene: val^{66}met. The reason for this focus is fully described in the introduction of this thesis. In sum, the interest in val^{66}met was fuelled by two studies that showed that this polymorphism has functional properties. Egan and colleagues (2003) showed a reduced activity dependent expression of BDNF in cultured hippocampal neurons (*in vitro*) that carried a met allele. These authors extended this finding by showing worse cognitive functioning and altered hippocampal memory activity in human met carriers as compared to val/val homozygotes. In a paper published in Science (2006), Chen and colleagues confirmed these findings (*in vivo*).

Based on these groundbreaking findings, variation at the BDNF val^{66}met locus has become one of the most influential models to study BDNF functioning and it is generally believed that the field benefitted by the identification of the presumed functionality of this polymorphism (Lu *et al.,* 2013). For human studies it seems particular interesting to assess variation at the val^{66}met locus in relation to several phenotypes because it is believed that variation at this locus mirrors individual (chemical) differences in BDNF functioningin the brain. In line with this presupposition are some human studies that apparently reproduce the animal findings (*e.g.,* phenotypic hallmarks of depression such as lower hippocampal volumes in met allele carriers as compared to val/val homozygotes [Pezawas *et al.,* 2004]).

In a series of three studies, we addressed the relevance of this polymorphism with regard to: BDNF serum concentrations, DSM-IV depression and anxiety diagnoses, depression- and anxiety symptom severity, cognitive functioning, and hippocampal functioning and morphology. As an important add-on, we incorporated trauma and stress exposure in our studies to model inter-individual differences in outcomes due to these factors and their potential interaction with BDNF val^{66}met. This is imperative for the reasons that: (**I**) strong inter-individual differences exist in the degree of how detrimental the effects of trauma/stress exposure on mental health are and this may be driven by individual genetic make-up (see for example Caspi and Moffitt, 2006) and (**II**) trauma/stress exposure is a central theme in the neurotrophin hypothesis (Duman and Monteggia, 2006).

The expectations were that established correlates of depression would be related to the genotypic variant that is associated with lower neurotrophic support (i.e., the met variant) particularly in the face of trauma- or stress exposure. Some of our explorations yielded results that were in line with this expectation. Many, however, also were not. Below these findings are discussed.

BDNF val^{66}met – trauma/stress exposure and serum BDNF concentrations

In **Chapter 7** we addressed whether variation at the val^{66}met locus influences serum BDNF concentrations. The main effects of exposure to childhood abuse (*i.e.,* sexual-, physical-, or emotional abuse exposure before the age of 16 years), recent negative life events (*i.e.,* stressful events such as a divorce in the year before measurements) and their potential cross-term interactions with val^{66}met were also assesed. Our focus on the cross-term interactions among BDNF val^{66}met and stress exposure followed specifically from studies that reported that met allele carriers are more vulnerable to the effects of stress exposure as compared to individuals who are homozygous for the val allele when considering depressive symptoms (Wichers *et al*., 2008), hippocampal volume (Gatt *et al*., 2009), and cognitive functioning (Gatt *et al*., 2009).

The well-powered and controlled study reported in **Chapter 7** rendered some interesting findings. First, in the absence of main effects of trauma exposure and val^{66}met it was found that the impact of childhood abuse on serum BDNF concentrations was dependent on variation at the val^{66}met. Specifically, in met carriers, trauma exposure was associated with reduced serum BDNF concentrations, whereas in the val/val group BDNF concentrations were even higher when trauma exposure was reported (*i.e.,* a cross-over effect). The BDNF reductions that were associated with childhood abuse in met carriers were linear in nature, so that BDNF concentrations were lowest in met carriers reporting exposure to multiple types of trauma. These findings follow the neurotrophin hypothesis to some extent. Yet it should be noted that they were not in total agreement, as no associations were found between being met carrier and higher depression severity or the presence of a DSM-IV depression diagnosis, also not when exposed to childhood abuse. Maybe the conjunct of the here observed effect on serum BDNF concentrations and on psychopathology is not mandatory in order for the neurotrophin hypothesis to be valid, but it would have strenghtened the model.

A second exciting finding described in **chapter 7** was that exposure to stressful events that occurred in the past year was associated with reduced serum BDNF concentrations. This effect was, in contrast to that of childhood abuse, independent of variation at the val^{66}met locus. This result directly replicates earlier findings (*e.g.,* Trajkovksa *et al*., 2008). The finding of lower serum BDNF concentrations following stress exposure also corroborate with a body of knowledge derived from animal studies (see for instance *et al.,* 2012) and obviously also with the neurotrophin hypothesis. Interestingly, the decreased serum BDNF concentrations following recent stress exposure were, as may be expected, associated with relatively high levels of depression symptom severity. It is tempting to link these two findings, but note that these results, remarkable as they may seem, are only correlation in nature. It further should be mentioned that although statistically significant, the effect was small as it only explained ~ 1 percent of the variance in serum BDNF concentrations.

Together the findings described in **chapter 7** suggest (notwithstanding considerations as the use of cross-sectional data) that a chain of events, commencing with gene-environment interactions, may lead to low serum BDNF concentrations. It would be interesting if longitudinal studies could further unravel the developmental trajectories towards psychopathology that follow trauma and/or stress exposure and whether these may run through individual genetic make-up and neurotrophic functioning.

BDNF val^{66}met – trauma/stress exposure and the hippocampal formation

In **chapter 8** we used functional and structural MRI techniques in order to test associations between the val^{66}met variant and the structure and function of the hippocampal formation, a critical brain structure in the pathophysiology of depression (MacQueen and Frodl, 2011). For similar reasons as provided previously, we took trauma- and stress exposure into account. The study yielded the following results. First, and in line with earlier studies, we find slightly smaller hippocampal volumes in carriers of a met allele relative to val/val homozygotes. This effect has been explained as being the result of abnormal intracellular trafficking and impaired activity secretion of BDNF in carriers of a met allele (Chen *et al*., 2006). Since atrophy of the hippocampus has also been associated with (early life) stress exposure and/or a having (had) a depressive episode (MacQueen and Frodl, 2011), it is crucial to exclude the possible confounding effects of these variables. In previous studies, stress exposure and depression diagnosis have largely not been taken into account (with the exception of Frodl *et al.,* 2007 and Gatt *et al.,* 2009). We did explicitly model these interactions. It turned out, however, that the association between the met allele and lower hippocampal volume was independent of trauma/stress exposure and current/lifetime depression. This null finding is at odds with the findings reported by Gatt *et al.* (2009), who found that the combination of carrying a met allele and being exposed to early life stress was associated with particular small hippocampal volumes (and a large number of other *hallmarks* of depression such as poor cognitive functioning). The observed discrepancy between the results of Gatt *et al*. (2009) and ours may be due to a broader definition of early life stress by Gatt and colleagues (2009) who included for example also *illness* as stressful event whereas we specifically focused on childhood abuse including physical, sexual, and emotional abuseIt remains unclear how this between-study difference could have led to a different pattern of results, assuming that neither one is due to chance. With regard to the latter it should be noted that a recent large-scale study (568 healthy participants; Gerritsen *et al.,* 2012) also could not replicate the findings by Gatt *et al.* (2008). The issue of non-replication will be discussed in a next part.

In addition to on average slightly reduced hippocampal volumes, we show in **chapter 8** that val^{66}met interacts with (emotional) word valence on hippocampal encoding activity. This effect is such that hippocampal related encoding activity is increased in carriers of a met allele when presented with negative words and not when presented with neutral or positive words as compared to val/val homozygotes. This

effect was not observed in other brain areas and seems to be consistent with some studies in which emotional stimuli were used (*e.g.,* Dennis *et al*., 2010 or Lau *et al*., 2010). Although intriguing, it is imperative to mention that, as in **chapter 7** (note that the sample in **chapter 8** is a sub-sample of the much larger sample that was used in **chapter 7**), despite effects on neurobiological measures (in this case brain morphology and neuronal activity) also in this study there were no corresponding effects of the same constellation of predictor variables on psychopathology outcomes (*e.g.,* depression diagnosis, illness severity). A critical point here is that this particular study with a total *N* of only 157 may have lacked the necessary statistical power to detect between-group differences that may be small at best.In addition, with regard to the absence of associations between hippocampal volume, hippocampal function, and memory performance, a recent review showed, in line with our findings, that the model: *'a bigger brain structure → greater brain response → better performance'* may not reflect reality (Eyler *et al*., 2010).

Notwithstanding the above, the **chapters 7**, **8**, and **9** also yielded some findings that were not in line with the expectations as they can be derived from the neurotrophin hypothesis. These inconsistent findings are the topic of the section that follows.

BDNF val^{66}met – inconsistent findings

I would like to start with the finding from **chapter 9** because the study in this chapter turned out to be a defining one.

Chapter 9 reports a systematic review and meta-analysis on the association between val^{66}met and total hippocampal volume. This study was undertaken because inconsistenties have been reported with regard to this association (see for instance the difference in outcomes between Szeszko *et al.,* 2005 and Dutt *et al.,* 2009). The potential influence of demographical, clinical, and methodological characteristics of studies was also assessed. Meta-analysis confirmed that carriers of a met allele had lower hippocampal volumes relative to val/val homozygotes, yet with a very small effect-size (d = 0.13, P = .02; k = 25, total N = 3,620). However, between-study heterogeneity in effect size estimates was substantial and this could not be explained by demographical, clinical, and methodological differences across studies. Funnel plot inspection and trim-and-fill estimations suggested evidence for publication bias and effect sizes decreased substantially over the years (Pearson's correlation coefficient on the relation between year of publication and effect-size was -0.54). When publication bias was taken into account the association between val^{66}met and total hippocampal volume was no longer statistical significant. A further striking finding was that all included studies were largely underpowered. Altogether, this shows that variation at the val^{66}met locus is not likely to account for individual differences in hippocampal volume but rather that the association is subject to a *winners curse*, with large effect sizes found in a few early studies and increasingly smaller effect sizes in later (better-powered) studies.

This finding does not stand on its self. When taking a close look at the *best evidence* in the current literature a trend becomes clear. Mandelman and Grigorenko for instance (2012) pooled the data on the association between val^{66}met and general cognitive ability, memory and executive functioning (k = 23, total N = 7,095) and found, despite promising initial studies (*i.e.,* Egan *et al.,* 2003), no association between val^{66}met and cognitive functioning. Another recent meta-analysis by Kambeitz and colleagues (2013) showed, when publication bias is taken into account, that the val^{66}met polymorphism has no effect on the neuronal systems underlying the encoding of information into episodic memory (hippocampal and para-hippocampal encoding activity; 16 comparisons N = 2,985). This finding also is in contrast to what was previously claimed (e.g., Montag *et al.,* 2009). A final example is a study by Gyekis and colleageaus (2013) showing, using the largest number of subjects to date, no evidence for an association of val^{66}met with the

diagnosis depression (*k* = 26, total *N* = 17,426). This is notable since previous meta-analyses (*e.g.,* Verhagen *et al.,* 2009) have suggested that the met allele was associated with a depression diagnosis.

The pattern is clear: the evidence for associations between BDNF val^{66}met and depression relevant phenotypes is waning. Based on this I conclude that the val^{66}met polymorphism has little, if any, prediction accuracy regarding depression related phenotypes. Another consideration in formulating this conclusion is that BNDF val^{66}met was not associated (again in spite of earlier evidence) with psychopathology outcome (**chapter 7** and **8**), serum BDNF concentrations (**chapter 7**), cognitive performance (**chapter 8**), nor with hippocampal volume (**chapter 9**). So, albeit knowing the (presumed) functionality of a polymorphism (through preclinical work) the studies in this thesis show (and a large literature from other groups as well) that this is not neccesarily associated with sampled outcomes in humans. Imperative for the interpretation of the above (and also for the earlier confirmative findings) is the recent finding that many genetic variant, deletions, and copy number variants are found in neuronal cells that do not correspond with those found in non-neuronal cells (McConnel *et al.,* 2013). Therefore, our findings regarding BDNF val^{66}met, that were based on the genotype of non-neuronal cells, may be limited in that the exact correspondence between these cells and neuronal cells is not known. This issue should be acknowledged in future (single-cell) studies.

Recapitulating our work on BDNF val^{66}met in relation to the neurotrophin hypothesis

Table 4 ↓ gives an overview of the findings in this thesis that regard BDNF val^{66}met and how these relate to the predictions from the neurotrophin hypothesis. Conclusions will follow in a next part.

Table 4. Summary of the research findings in this thesis on BDNF val^{66}met and how they fit with the neurotrophin hypothesis (confrimative versus non-confirmative)

Confirmative
1. Carriers of a met allele seem to be more *vulnerable* with regard to childhood trauma exposure when serum BDNF concentrations are taken as an outcome (**chapter 7** [*N* = 1,435]).
2. Carriers of a met allele locus have somewhat lower hippocampal volumes as compared to val/val homozygotes (**chapter 8** [*N* = 157])
3. Carriers of a met allele locus show higher hippocampal activity in response to words of negative emotional valence as compared to val/val homozygotes (**chapter 8** [*N* = 157]).
Non-confirmative
1. Variation at the BDNF val^{66}met locus is not associated with serum BDNF concentrations, depression diagnosis, and depression symptom severity (**chapter 7** [*N* = 1,435]).
2. Variation at the BDNF val^{66}met locus is not associated with cognitive performance and the brain functioning (**chapter 8** [*N* = 157]).
3. Lower hippocampal volumes are not associated with carrying a met allele at the BDNF val^{66}met locus [chapter 9 [*N* = 3,620]).

The neurotrophin hypothesis and our work – recapitulating

Before I will start to contemplate on the strengths and limitations of the studies reported herein and state my conclusions, please see **Table 5 ↓** (next page) for a summary of all the findings in this thesis and how they relate to the predictions from the neurotrophin hypothesis is provided.

Table 5. Summary of research findings and how they fit with the neurotrophin hypothesis (confrimative versus non-confirmative)

Confirmative
1. Serum BDNF concentrations are low in depressed patients relative to healthy controls (**chapter 4** [*N* = 923] and **chapter 5** [*N* = 5,203]).
2. Serum BDNF concentrations are normalized in the course of depression remission (**chapter 4** [*N* = 1,080] and **chapter 5** [*N* = 4,204]).
3. Serum BDNF concentrations are normalized in the course of antidepressant treatment (**chapter 4** [*N* = 1,080]; **chapter 5** [*N* = 4,204]).
4. A larger increase in serum BDNF concentrations is associated with a larger decrease in depressive symptoms over the course of antidepressant treatment (**chapter 5** [*N* = 1,422])
5. Serum BDNF concentrations are low in female patients with an anxiety disorder relative to female controls (**chapter 6** [*N* = 499]).
6. Exposure to recent stressful events is associated with lower serum BDNF concentrations (**chapter 7** [*N* = 1,435]).
7. Carriers of a met allele seem to be more *vulnerable* with regard to childhood trauma exposure when serum BDNF concentrations are taken as an outcome (**chapter 7** [*N* = 1,435]).
8. Carriers of a met allele locus have somewhat lower hippocampal volumes as compared to val/val homozygotes (**chapter 8** [*N* = 157])
9. Carriers of a met allele locus show higher hippocampal activity in response to words of negative emotional valence as compared to val/val homozygotes (**chapter 8** [*N* = 157]).
Non-confirmative
1. Serum BDNF concentrations are low in the early remission phase of depression as compared to the active phase of depression (**chapter 4** [*N* = 541])
2. Serum BDNF concentrations are normalized in the course of treatment with an antidepressant but this is not associated with remission (**chapter 4** [*N* = 421]).
3. Several classes of equally effective pharmacological antidepressants are differently associated with serum BDNF concentrations (**chapter 4** [*N* = 421]). [A]
4. Clinical characteristics (most notably depression severity) are not associated with serum BDNF concentrations (**chapter 4** [*N* = 541]).
5. The differences in serum BDNF concentrations as a function of diagnostic and treatment status are overestimated and are of a small effect-size at best (**chapter 5** [*N* = 9,484]).
6. Serum BDNF concentrations are normal in male patients with an anxiety disorder relative to male controls (**chapter 6** [*N* = 276]).
7. Childhood trauma exposure is not associated with serum BDNF concentrations (**chapter 7** [*N* = 1,435]).
8. Variation at the BDNF val^{66}met locus is not associated with serum BDNF concentrations, depression diagnosis, and depression symptom severity (**chapter 7** [*N* = 1,435]).
9. Variation at the BDNF val^{66}met locus is not associated with cognitive performance and the brain functioning (**chapter 8** [*N* = 157]).
10. Lower hippocampal volumes are not associated with carrying a met allele at the BDNF val^{66}met locus [chapter 9 [*N* = 3,620]).

[A] Note that the possibility exist that different types of antidepressants may be clinically efficacious through different mechanisms (Mann 2005) and that therefore this finding is not neccesarly non-corfirmative with te neurotrophin hypothesis.

Conclusion

What is the final word on this? I do not think that we are on the verge of understanding depression through peripheral BDNF measurements or genetic variants that are supposed to be associated with neurotrophic functioning. The lack of universality of findings on BDNF alternations in depression that is brought forward in this thesis (and also by other research groups in recent years) suggest that attributing behavioral differences to peripheral BDNF parameters and genetic variants is overreaching. There is simply too much clinical data that do not corroborate, or are even tangential to, the predictions of the neurotrophin hypothesis. Of course, and taking for instance the heterogeneity of depression into mind, inconsistencies do not necessarily reject the neurotrophin hypothesis for all depressed patients. Besides, some predicted associations from the neurotrophic model appear to be established (*e.g.,* abnormally low serum BDNF concentrations in the depressed state). Nonetheless, in these instances the meaning of them often is not that clear (*e.g.,* reverse causation in which low neurotrophic support does not endanger a person to become depressed but rather are a consequense of being in the depressed state). In fact, reverse causation largely is my theory.

Furtermore, what this thesis illustrates clear is the value of well-powered studies, as it shows that some of the core observations on which the neurotrophin hypothesis rests are less evident, and sometimes even absent, when well-powered studies are used. This was particularly evident in our work on val^{66}met.

So, in my view the conventional wisdom that existed at the time of the start of my PhD tract that peripheral BDNF parameters and genetic variants are relevant in the pathophysiology of depression is too far fetched. In fact, from the above I conclude, whilst taking limitations into account and acknowledging that the results herein are largely contingent upon peripheral measurement that the neurotrophin hypothesis should no longer be credited in its original form.

Methodological (and other) considerations and future work

Strengths

The studies that form the heart of this thesis have salient strengths. First of all, in basically each individual study, results are derived from a large single sample or from data that come from multiple studies and together add up to a large sample. This safeguards against false positive- and negative findings and provides effect-size estimates that are accurate with regard to their magnitude (Ioannidis 2005). The proof of this principal became evident in the pooled effect-sizes that were derived from the meta-analyses that we performed, as these converged closer to those that were reported in studies that used a relatively large sample size as compared to those studies that used a relatively small sample size. A second notable strength of our work is that most analyses were adjusted for a range of possible confounding factors and that we were able to perform subgroup and moderation analyses. This allowed us to infer on (largely) independent associations, which increases, although not guarantees, the likelihood of valid findings. Validity was also achieved through the use of standardized diagnostic tools to assess current and lifetime psychopathology and the use of a control group (although the latter not necessarily eliminates all possible confounders; Prasad and Jena, 2013). Furthermore, for the interpretation of our findings we did not solely rely on *P*-values (Johnson 2013) as, where appropriate, we reported effect-size estimates and their respective confidence levels as well.

Notwithstanding strengths in design, method and reporting, I am well aware of the limitations that carry our work. The main limitations, besides those already mentioned, are discussed below.

Limitations

Table 6 ↓ lists the main limitations of the work in this thesis. These limitations are discussed in the section that follows.

Table 6. Limitations of our studies by chapter

Limitation	Because	Chapters
Cross-sectional data	Not sufficiently persuasive to prove causality	2-9 (mostly)
Non-random allocation	Not sufficiently persuasive to prove causality	2-9 (mostly)
Generalization of findings	Our study findings do not (directly) generalize to all populations	2-9 (mostly)
A multitude of tests and power	Large data-sets do not protect against multiple testing	2-9 (mostly)
	Some effects may be so small that they are hard to detect even when using a large sample	
	Some (underpowered) *post-hoc* tests may have yielded false positive- or negative findings	
Reliability, validity, and error	The measures that were used are not 100 percent reliable and valid	2-9 (mostly)

Cross-sectional data

A limitation of most of the work in this thesis is that it relies on data that were collected in a single wave. This is a limitation because data that is gathered in such a manner does not allow for conclusions that delineate the time course of event, let alone matters of causality. Take for instance our finding that serum BDNF concentrations are abnormally low in the depressed state. This finding could be explained so that a low expression of BDNF predisposes or endangers a person to an episode of depression (*i.e.,* the temporal precedence of a cause–effect relation). Indeed, this could be so. However, we cannot infer from our data that alternate explanations are false. For instance, it could be that the low serum BDNF concentrations in the depressed state are a consequence of being depressed (as has been discussed above). Note though that a lack of clarity with regard to temporal precedence is not a limitation for all cross-sectional findings that we reported. Take for instance the correlation between the amount of ambient sunshine and serum BDNF concentrations (**chapter 3**) where the presumed *cause* can be placed before the *event*, as it is not very likely that BDNF in blood causes the sun to shine. The findings regarding stress exposure and serum BDNF concentrations can be interpreted along a similar line. Yet, also for these particular cases, repeated sampling on each individual would have been more persuasive.

Non-random allocation

Another obvious limitation is that due to the epidemiological nature of the NESDA data, of which we made extensive use, none of the participants were randomly allocated to the conditions, such as medicated versus non-medicated. Therefore, our work lacks the experimental nature that is needed for causal inferences. Take for example our finding that distinct classes of antidepressants seem to have a differential effect on serum BDNF concentrations (**chapter 4**). The patients in this study were not randomly assigned to the particular antidepressant condition. Hence, *a priori* differences may have existed between persons who used a different kind of antidepressant. For instance, persons who were treated with TCA's, which is not a *first-choice* antidepressant, may have represented a clinically distinct group, consisting of a large number of non-responders on treatment with SSRI's, which typically is a *first-choice* antidepressant (Mann 2005). Thus, what we labeled as being an antidepressant-specific effect on serum BDNF concentrations may actually have been the effect of being a non-responder to treatment with a SSRI. Although we did test for a great number of possible confounders and actively explored alternative explanations, still some between-group differences may have gone undetected. Thus, because of non-random allocation, our work does not prove causality.

Generalization of findings

Other limiting factors regard the exact extent to which it can be generalized to the population at large or to specific subgroups within the population. The clinical scope of the work in this thesis is broad, as the age range of the NESDA sample is wide (18 to 65 years of age), and NESDA includes patients from several sources (*i.e.,* primary care and out-patient clinics). Notwithstanding this, in the NESDA sample no children/adolescents or elderly persons are included and the persons with depressive and anxiety disorders are all outpatients with in general modest levels of symptom severity. Also, most of the participants that were enrolled in our studies are from a Caucasian descent. So, generalizations from our findings to the young and the old, the severely ill (*e.g.,* patients who receive intra-mural care), and to persons who are not from a Caucasian background may not be straightforward. This also holds for our findings that were derived from meta-analyses, since the studies that were included also enrolled mostly persons from a Caucasian background and if they were patients, then their symptom severity was in general not that high.

Multiple testing and power

In an earlier part of this section, the use of large samples was heralded as a strong point of our work. A large sample indeed comes with advantages. However, it does not protect against the testing of a multitude of hypotheses. Given that in this thesis a substantial number of hypotheses were tested (using a single large data set) our work may have yielded some false positive findings. Second, some effects are so small that they cannot be reliably detected even when large numbers of subjects are included. The null-findings that were derived from our studies on the presumed relationship between val^{66}met and cognitive functioning (see **chapter 8**) may be a good example of this.

While considering this, statistical power is just as important to take into account when faced with positive findings (Christley 2010). Again, although overall we performed analyses using comparatively large sample sizes, at times we performed sub-group analyses that may have lacked sufficient statistical power. Likewise, the meta-regression analyses, reported in the **chapters 5** and **9**, may have been underpowered since these were based on the rather small number of studies. Our search for moderators therefore may have yielded significant associations that have different effect-sizes or actually are non-existing.

Measurement: you can never have enough precision

We measured, analyzed and concluded on BDNF concentrations in serum derived from peripheral veins. Although there are inherent advantages to this method (*i.e.,* easily accessible, only minimal invasive, and reliable with regard to intra- and inter assay variability) some points of concern should be stated.

First of all, an assumption that we had is that peripheral BDNF measurements reliably mirror the amount of BDNF in the brain. The data that underlies this assumption rely for the larger part on positive correlations between BDNF concentrations in the central nervous system and the periphery (Klein *et al*., 2010) and active transport of BDNF through the blood-brain barrier (Pan *et al*., 1998) that have been shown in non-human animal studies. Furthermore, some rodent studies have shown that peripheral administration promotes the regeneration of spinal cord injury (Krishna *et al.,* 2013) and has an effect on depressive-like behavior (Schmidt and Duman, 2010). However, there is no clear consensus on this issue and criticism and uncertainty remain. There are good reasons for this. One, in the brain, the expression of BDNF is locally and time specific (Bennet and Lagopoulos, 2013). Animal studies have shown, for instance, that antidepressant treatment increases the expression of BDNF in some brain regions (*e.g.,* the ventral tegmental area; Taliaz *et al*., 2012) but not in others (*e.g.,* the hippocampus; Lanz *et al*., 2012; Taliaz *et al*., 2012). A second reason is that there are complexities in assigning the exact sources of BDNF in peripheral tissues (Bejot *et al*., 2011). The *brain-derived* part in the name BDNF suggests that all BDNF that is active in an organism has its origin in the brain. This however is at least a little misleading (Gass and Hellweg, 2010; R Hellweg, personal communication, 2013; B Bus, personal communication, 2009 through 2013) as several types of immune-, smooth muscle-, and endothelial cells serve as sources of BDNF as well (Karege *et al*., 2002). Thus, the BDNF concentrations that we measured are likely not to have come from the brain for a 100 percent. In fact, given that serum BDNF concentrations are much higher in serum as compared to that in cerebro spinal fluid (> a 1000 fold) they may largely reflect peripheral synthesis (Pillai *et al.,* 2010). A consequence of this is that alternations in peripheral BDNF may not reflect (disturbed) central pathways but epiphenomenon of some other physiological or behavioral- and/or peripheral process that is not necessarily related to central BDNF functioning (as has been discussed above).

So, the serum BDNF measures may not more than a summed-up net, crude parameter of central BDNF functioning. Besides, some other issues regarding the measurement of serum BDNF should be acknowledged. This will be done below.

Serum BDNF concentrations versus other peripheral BDNF parameters

BDNF concentrations in serum are just one of several peripheral measures to gauge on neurotrophic functioning in the brain. Other available non-invasive options include BDNF concentrations in whole blood, blood plasma, and blood platelets. Since there are some studies that assayed a multitude of these parameters there is knowledge on how these parameters relate. In general, studies report statistically significant, yet mostly modest associations among these measures (*e.g.,* correlations between plasma and serum BDNF concentration in the range $r = 0.21$ and $r = 0.26$ (Terracciano *et al.,* 2011; Jeon *et al*., 2012) to $r = 0.71$ (Yoshimura *et al*., 2010)]. Thus, the measures that are used in the literature to gauge on the process of neurotrophic functioning in the brain relate, but far from perfect. Our findings derived from serum therefore are limited in scope in that they cannot be directly generalized to other peripheral BDNF parameters. Besides, this raises the question which parameter serves best as a mirror for neurotrophic action in the brain. Some authors have brought forward that leukocyte BDNF mRNA content, because of its short half-life, could more closely reflect central BDNF dynamics (Gass and Hellweg, 2010) and therefore perhaps may be less subject to (peripheral) confounding factors. In addition, it has been argued that a combination of peripheral BDNF indices may have advantages above a single one. Assessing both platelet and serum BDNF concentrations could be in particular relevant. Blood platelets store BDNF and release BDNF during the clotting process and by agonist simulation (Rosenfeld *et al*., 1995; Fujimura *et al*., 2002). Therefore it could be that inter-individual differences in serum BDNF concentrations are mediated by a lower activity of blood platelets caused by medications (notably here antidepressants) or pathological conditions (notably depression; Karege *et al.,* 2002).

Pro- versus mature BDNF

Besides the limitations of measuring in the periphery, there are some other drawbacks regarding the methods that we used to quantify BDNF. One is that the ELISA kit that was used in our studies could not make the distinction between the pro- and the mature BDNF variant (Lu *et al*., 2005). Thus, what we have quantified are total BDNF concentrations in serum without any regard to whether it was the pro- or the mature form. Given that the two BDNF variants are functionally different (see the introduction part for this), it would have been interesting to study whether pro-mature BDNF ratios differed, for instance, among diagnostic groups. The antibody that is sufficiently specific to make this distinction, however, was developed only recently by Yoshida *et al*. (2012a) and therefore not applied in the studies that make up this thesis.

Between-study differences and the golden standard

A final disadvantage is that large between-study differences are reported in mean serum BDNF concentrations. This has even been shown for BDNF concentrations that are assessed by the same research group, among similar diagnostic groups, using the same ELISA kit (*e.g.,* Karege *et al*., 2002 and 2005: mean serum BDNF = 22.6 ± 3.4 versus 10.1 ± 2.3 respectively). These differences probably are the result of different laboratory procedures and for within-study comparisons and meta-analyses they are not likely to constitute a limiting factor. An unfortunate consequence however is that there is no such thing as an accepted reference value that defines an individual BDNF value to be high or low. Because of this, only within-study differences can be interpreted reasonably. Standardization of measurements would be of great value here.

Summarizing the previous section, as with basically all constructs, the ability to conclude on a construct depends largely on how well the construct can be measured. Serum BDNF concentrations can be reliably measured, yet with error and with noise. Besides, the correspondence between peripheral and central

BDNF functioning is far from clear and therefore the meaning that can be assigned to (largely all) peripheral measurements is only limited.

Future work – what is worth studying and what is worth changing?

In the part that follows I discuss some options to overcome the limitations that are sketched above. These options are listed in **Table 7 ↓**.

Table 7. Areas of future interest

I. Acquire mechanistic understanding on what exactly alters neurotrophic functioning in depression
II. Single studies versus teamwork and large scale data-sharing
III. Measure and study beyond single BDNF parameters and use within-subject data
IV. Present convergent evidence from multiple research levels (*e.g.,* man and mice data in conjunct) and leave broad diagnostic categories

Mechanistic understanding

Now, and despite large interest, there is no consensus on what exactly causes altered neurotrophic functioning in depression, let alone whether it is of functional significance for (mental) health. Learning about this should be the greatest aspirational goal for the field because based on such knowledge the question whether pathological processes or epiphenomena are at play could be answered. From our studies it appears that the axiom that to depression related alternations in BDNF expression are due to trauma- or stress exposure (Duman and Monteggia, 2006) likely does not hold. Probably the relation is more complex and moderated by other factors (see **chapter 7**). Besides, there are hints on mechanisms other than stress-exposure that may regulate altered neurotrophic functioning in the depressed state. Some of these are also discussed in this thesis (*e.g.,* menopausal stage and estrogen expression). Additionally, although not empirically pinned down, I formulated two explicit hypotheses that could thrive inter-individual differences in serum BDNF concentrations in depression and in the course of treatment for this illness (*i.e.,* oxidative stress and [to depression and treatment related] changes in body-weight). Note that these hypotheses rather suggest reverse causation (*i.e.,* depression → low BDNF instead of low BDNF → depression).

Single studies and ultimate answers -- teamwork matters

In the literature it is common practice to report single study findings. For several reasons I wish to argue that the relevance of future efforts would greatly increase when other approaches were used.

Most importantly, the ever-expanding individual study results should be placed in the quantitative body of knowledge that already exists. The need for integration is bigger than ever. Data integration is important for the reason that *single studies do not provide ultimate answers*. See for instance **chapter 5** where we through data integration show that serum BDNF concentrations are not associated with the symptom severity of depression, whilst this belief initially existed (Karege *et al.,* 2002). A similar example can be found in **chapter 9** where we show, also in contrast to what was generally believed (see *e.g.,* Pezawas *et al.,* 2004), that the val^{66}met polymorphism is not associated with hippocampal volume. A data-sharing network could be the approach to answer, with rigor, many of the outstanding questions. Actually and since I truly belief in it, I am trying to launch such a project. Wide support is necessary for this, so I sought and am seeking international collaboration, with among others, Brisa Fernandes (Hospital de Clinicas, Porto Alegre, Brasil), Maryna Polyakova (Max Planck Institute, Leipzig, Germany), Kenji Hashimoto (Chiba University,

Chiba, Japan), and Rainer Hellweg (Charite University, Berlin, Germany) to come to this end. Evidently a dating-sharing network (of existing data) comes at low costs.

Beyond a single BDNF measurement

The literature to date, obviously including our own work, largely materializes on single cross-sectional measurements. Instead I would like to promote to: (**I**) measure and study beyond single BDNF measurements, (**II**) gather longitudinal (instead of between-subject) data, and (**III**) provide convergent evidence.

The common practice nowadays is to extract a single BDNF parameter from blood whilst other hormones, neurotransmitters, and receptor systems are not taken into account. This is problematic because herewith those factors that may interact with BDNF, and in theory could explain observed associations, are neglected. My eyes therefore are on studies that in conjunct to BDNF measure for instance the enzymes that convert pro-BDNF to mature BDNF or cortisol-, tryptophan-, and serotonin blood levels. A particular good example this is the recent study by Zhou *et al.* (2013) in which pro- and mature BDNF concentrations alongside their respective receptor systems: p75 and Trk-B, were assessed in serum and lymphocytes. The data from this study showed the welcome evidence that proBDNF and p75 receptors were lower in depressed patients as compared to healthy controls whereas the opposite was observed for the mature BDNF variant and its receptor Trk-B.

Next, studies should rely less on data that are collected in a single wave but instead on within-subject data. This is a more appropriate manner since it excludes a large amount of between-subject variance and an accompanying increase in the possibility to detect meaningful associations. In this thesis an example for this can be found in **chapter 5** where we show that effect-size estimates are largest when they are derived from (pure) within-subject designs. Related, it is desirable that future studies should actively control for relevant confounders (see **chapter 2** and **3**) and are sufficiently powered (see **chapter 5** and **9** for some recommendations on this).

An ideal: convergent evidence

What for me represents a general low-point in the literature is that the preclinical and clinical work on the neurotrophin hypothesis disconnect: the first uniformly reports a causal role for BDNF in the development of depressive-like behavior whereas the latter reports many null findings (including, importantly, from meta-analysis [*e.g.,* the **chapters 5** and **9**] and many other examples, *e.g.,* Kambeitz *et al.* [2012] or Dodds *et al.* [2013]). There are clashes: preclinical researchers take the stance that clinical workers do not measure the right BDNF parameters and clinical workers the one that preclinical workers do not measure depression or manipulate BDNF functioning with too much rigor (*e.g.,* completely knock it down or overexpress it a manifold of times). This leaves the neurotrophin hypothesis as a theory beyond testability. Changing cultures is necessary here.

It seems hard to directly weigh the relevance of the preclinical versus the clinical evidence. In humans the knowledge on neurotrophic functioning is largely contingent upon peripheral parameters (except maybe the studies that focus on val66met, imaging- and *post-mortem* studies). A salient detail here is that the one study that cdme closed to neurotrophic functioning in the human brain (on the amount of neuronal proliferation) by Reif *et al.* (2006) reports negative results. Preclinical studies, instead, measure BDNF in central tissues and they provide more spatial (and temporal) precision. Such studies are indispensable (albeit maybe they come with little pathological validity [Krishnan and Nestler, 2010]). It can therefore be suggested that the methods that are used in the greater part of the human studies are just not the right ones. Taking this stance should push me to reframe the title of my thesis into something like: *will the*

neurotrophin hypothesis with its predictions on depressive disorders in humans sparkle on, long after the glitter of the firework is gone? But no, this is too long of a title (Mentink A. 2013, personal communication). Besides, I am interested in human depression *per se* and there are claims that preclinical studies are too lofty and oversold (*e.g.,* manipulations that lack ecological validity) with regard to the human template they model (Couzin-Frankel 2013). Yet, progress in understanding the neurobiology of depression is contingent upon a combination of preclinical models, human cellular models and human biological studies (Hyman 2014). Besides, critical is the research evidence that comes from multiple levels (see for instance Tripp and colleagues [2013] for a good example and also that from our group, in a recent collaborative work with Maryna Polyakova (Max Planck Institute Leipzig, Germany).

SUMMARY AND CONCLUSIONS

The role of BDNF in Depression

Will the neurotrophin hypothesis sparkle on, long after the glitter of the firework is gone?

BACKGROUND

Neurotrophic support is ubiquitous in the brain where it is believed to be essential for the normal functioning of neuronal plasticity, memory and learning. The neurotrophin Brain-Derived Neurotrophic Factor (BDNF) is the main mediator of neurotrophic support and it has been stated repeatedly that by understanding it's regulation, the understanding of several psychiatric conditions can be increased. According to the neurotrophin hypothesis, depressive disorders arise from aberrant neurotrophic support by BDNF in brain areas that regulate emotion. Over the years, this hypothesis has gained steady steam. Furthermore, there is ground for the belief that peripheral measures (notably BDNF concentrations in blood) and certain genetic variants (notably BDNF val^{66}met) can serve as windows for neurotrophic functioning in the brain).

However, amid a lot of excitement, uncertainty regarding the predictions of the neurotrophin hypothesis remains. Sources of uncertainty are a lack of knowledge on the basic determinants of serum BDNF concentrations and unanswered clinical questions. In this thesis I tried to provide a more refined model of (peripheral) neurotrophic functioning in depressive (and related) disorders by addressing these two sources of uncertainty.

The empirical data that forms the hart of this thesis and a discussion on it are presented in the foregoing chapters. A detailed summary will be presented in the following section.

RESULTS

Below, the results of our empirical studies are presented (by chapter) alongside the significance that I believe that they may have. The first aim of this thesis, to delineate the basic determinants of serum BDNF concentrations, is reported in **chapter 2** and **3**. The **chapters 4** through **9** answer clinical questions regarding the neurotrophin hypothesis.

PART I: Determinants

Chapter 2 provides a detailed description of the basic determinants of serum BDNF concentrations. It shows, in persons who were untreated with antidepressants and free of a current psychiatric illness, that a non-fasting state at the time of blood draw, later measurement on the day, longer sample storage, and being a binge drinker all were associated with attenuated serum BDNF concentrations. This was in contrast to smoking and living in an urban area, which both were associated with increased BDNF concentrations. Finally, older subjects had higher serum BDNF concentrations, but this mostly applied to women (*i.e.,* a gender-age interaction effect).

The significance of this paper is that it sketches the basic determinants of serum BDNF concentrations. Herewith, it provides an improved base to understand inter-individual differences in serum BDNF concentrations and knowledge that is essential in preventing erroneous inferences from data.

In **chapter 3** we studied seasonal entrainment of serum BDNF concentrations. Analyses by month of sampling (monthly *n*'s all > 196) showed pronounced seasonal variation. Serum BDNF concentrations increased linearly over the spring-summer period (*i.e.,* equinox vernal) and decreased linearly over the autumn-winter period (*i.e.,* equinox autumnal). Explorative analyses showed that the natural length of day

and the number of ambient sunshine hours (major triggers to entrain seasonality) in the weeks prior to blood withdrawal correlated with serum BDNF concentrations.

These findings add to the literature as they provide avenues to understand those factors that regulate BDNF expression. Besides the findings reported herein are of vital importance in the design- and evaluation of studies on BDNF.

PART II: the neurotrophin hypothesis of depression

In the **4th chapter** we advance the understanding of the associations between serum BDNF concentrations and depression. Using data on 962 depressed patients, 700 remitted depressed persons and 382 healthy controls we found serum BDNF concentrations to be low in antidepressant-free depressed patients relative to controls and to depressed patients who were treated with an antidepressant. Serum BDNF concentrations of fully remitted persons were comparable to those of healthy control subjects. Analyzing the sample of antidepressant-free depressed patients showed that BDNF concentrations were unrelated to the core clinical features of depression such as its severity.

This paper reveals that low serum BDNF concentrations are a state characteristic of depression that normalizes in the course of natural remission and antidepressant treatment. Critically is that in this paper we show that the effect-sizes on these associations are small and that normalization of serum BDNF concentrations is not necessarily associated with a relief of depressive symptoms.

Chapter 5 reports the findings of meta-analyses on differences in serum BDNF concentrations in antidepressant-free depressed patients versus healthy control subjects and antidepressant-treated depressed persons. The paper shows low serum BDNF concentrations in 2,384 antidepressant-free depressed persons relative to 2,982 healthy controls and to 1,249 antidepressant-treated depressed patients. When publication bias was accounted for, these effect-sizes became small to medium at best. This paper further shows, in contrast to prior belief, that serum BDNF concentrations and the symptom severity of depression are not related.

This paper is noteworthy, not in that it confirms that alternations in serum BDNF concentrations appear to be peripheral manifestations of depresion but that it shows that the evidence for this is slimmer as was initially thought. An important implication of this message is that serum BDNF concentrations probably are of little clinical use.

In **chapter 6** we evaluated serum BDNF concentrations in 393 patients with an anxiety disorder and in 382 healthy control subjects. We found no overall differences in serum BDNF concentrations among patients and controls. A gender-diagnosis interaction on serum BDNF concentrations however was detected indicating that female patients with an anxiety disorder had lower serum BDNF concentrations relative to female controls. This was not observed in males. Serum BDNF concentrations were unrelated to the clinical characteristics of anxiety.

Anxiety disorders mimic depression to a great extent, so it was expected that serum BDNF concentrations would be low in patients with such an illness. Except for somewhat lower serum BDNF concentrations in female patients, this paper does not confirm the expectation. This may suggest that BDNF is involved in the pathophysiology of anxiety in women or, not unlikely, that the somewhat lower serum BDNF levels in anxious women are a female specific artifact of being anxious.

The **7th chapter** addressed the presumed effect of BDNF val^{66}met on serum BDNF concentrations and whether it, if there, is conditional upon exposure to childhood trauma or other forms of stress. Overall, met

carriers had reduced serum BDNF concentrations when exposed to childhood abuse. This effect was dose-dependent. Moreover, when not exposed to childhood abuse, met carriers had higher BDNF concentrations than val/val individuals (*i.e.,* a val/met-abuse interaction effect). Exposure to recent life events was associated with a small decrease in BDNF concentrations.

Trauma- or stress exposure is, according to the neurotrophin hypothesis, the axiom that prevails in explaining depression related alternations in BDNF expression. This paper largely does not confirm this idea. The paper does report a val^{66}met – childhood trauma interaction effect on serum BDNF concentrations. The extent to which this interaction may be important (on various levels of functioning) remains elusive.

In **chapter 8** we unravel whether the volume and functioning of the hippocampus and cognitive performance are related to variation at BDNF val^{66}met. Structural and functional MRI data on depressed and/or anxious patients and healthy control subjects was used to elucidate this. Our results showed slightly smaller hippocampal volumes in carriers of a met allele relative to val/val homozygotes. For hippocampal encoding activity we find a val^{66}met–word valence interaction such that carriers of a met allele showed increased levels of activity in response to emotional laden words.

This paper furthers the understanding of the association of BDNF val^{66}met with hippocampal volume/functioning and cognitive performance. Critically, it takes trauma/stress exposure into account. We find a small effect of val^{66}met on hippocampal volume and that childhood abuse accounts for individual differences in hippocampal encoding activity. This latter effect manifests itself differently as a function of val^{66}met. These findings, although in need for replication, raise the question whether met carriers show abnormal brain response on emotional laden stimuli. This message comes with the notion that no behavioral effects were observed alongside the neurobiological differences.

Chapter 9 contains a review and meta-analysis on the association between BDNF val^{66}met and hippocampal volume. The results showed that carriers of a met allele had somewhat lower hippocampal volumes relative to val/val homozygotes. Between-study heterogeneity in effect size estimates was substantial and this could not be explained by demographical, clinical, and methodological differences across studies. We found strong evidence for publication bias and effect sizes decreased substantially over the years. This all led to the conclusion that lower hippocampal volumes are not a genuine biological effect of the met allele but rather a methodological artifact.

The finding of low hippocampal volumes in met carriers has become a pillar for the neurotrophinn hypothesis (total citations for the first paper on this association [Pezawas *et al.,* 2005] is > 500). The meta-analysis on this subject however shows that this association is non-existent and probably an artifact due to the use of underpowered studies.

METHODS

Before I present the highlights of the discussion and my conclusions, I shortly present the main strengths and limitations of the methods that were used in this thesis because these need to be reflected upon when interpreting the way in which I gave meaning to the findings herein.

A salient strength is that basically all studies in this thesis were extremely well powered. Herewith this thesis provides reliable effect-size estimates (Big Data Notable Fidelity). Another strength is that a high level of validity was achieved through adjustment for potential confounding factors and through subgroup- and moderation analyses. Besides, I actively sought to determine how reliable our research findings were by conducting meta-analyses.

Notwithstanding this, I am well aware of the limitations of the studies in the prevailing thesis. First, our conclusions are contingent on peripheral measures to gauge on the central process of neuronal plasticity. Second, this thesis mostly elaborated on cross-sectional data and in none of the studies the subjects were randomly allocated to the conditions in which they were. Thus the reported findings are not sufficiently persuasive to prove causality. Finally, the findings of this thesis do not (directly) generalize to all populations, notably not to the young, the old and the severely ill.

DISCUSSION

This thesis accomplishes two things: (**I**) it outlines the determinants of serum BDNF concentrations and (**II**) it resolves some important clinical questions regarding the neurotrophin hypothesis. Together these accomplishments have significant methodological and theoretical implications that are summarized below. I will start with the findings on the determinants of serum BDNF concentrations.

Part I: determinants

Gaining detailed insight in determinants or the confounding structure of certain traits is the best strategy to avoid spurious associations and therefore worthwile. The knowledge on this issue was only rudimentary when I started this thesis trajectory back in 2008.

In line with a conceptualization of serum BDNF concentrations as being dependent on a complex myriad of factors, a long-list of variables was discovered that systematically showed association with serum BDNF concentrations. On this list were many variables that could have easily confounded the results of studies that test the predictions from the neurotrophin hypothesis. An example that illustrates this well is seasonality. It is established that the prevalence of depression is higher in the winter- as compared to the summer months (Lewy *et al.,* 2006). In **chapter 3** I report profound seasonality in serum BDNF concentrations. Together this suggests that controlling for season could change the magnitude of some associations with a prime interest of the neurotrophin hypothesis, for instance the difference in serum BDNF concentrations among depressed patients and healthy controls. This turns out to be so, as the data in **chapter 3** shows that the effect-size on this association is attenuated by about 40 percent when seasonality is taken into account.

In this thesis, our group has begun to make progress in understanding the factors that systematically influence serum BDNF concentrations. The focus was on methodological issues, that is: *how to avoid confounding*. This was achieved as the findings that are reported in **chapter 2** and **3** invite for a perspective on BDNF related research in which the basal determinants and seasonality are engrained. Herewith, we contribute timely to the literature

Part I: the neurotrophin hypothesis

The **chapters 4** through **7** set out to explore serum BDNF concentrations in relation to psychiatric illness, notably depression. Explicitly tested was whether: (**I**) patients with depressive disorders (and related conditions) consistently exhibit abnormally low serum BDNF concentrations, (**II**) serum BDNF concentrations are related to the characteristics of depression (*e.g.,* the severity of symptoms), (**III**) antidepressant use accounts for variance in serum BDNF concentrations, and (**IV**) etiological risk factors for depression (trauma and stress exposure) account for variance in serum BDNF concentrations.

Serum BDNF concentrations - confirmative findings

In line with what was expected, **chapter 4** and **5** show that serum BDNF concentrations are low in antidepressant-free depressed patients relative to controls and to depressed patients who were treated

with an antidepressant. The data from **chapter 4** and **5** further indicate that serum BDNF concentrations of fully remitted persons are comparable to those of healthy controls. So, low serum BDNF concentrations are a state characteristic of depression: evident during the depressed state and normalized in full remission. Also in line with what was expected is that serum BDNF concentrations normalize in the course of antidepressant treatment. All these findings are robust since they were derived from the largest (and most reliable) single study (**chapter 4**) and confirmed by meta-analyses (**chapter 5**). Extending the neurotrophin hypothesis we find that female patients with an anxiety disorder have lower concentrations of BDNF relative to female controls and to male patients (**chapter 6**). This suggests that BDNF might be involved in the pathophysiology of anxiety in women. Finally, and also conform expectations was that exposure to recent life events such as being fired (in general an etiological risk factor for the development of a depressive episode), was associated with a (small) mean decrease in serum BDNF concentrations (**chapter 7**).

Despite that these findings were confirmative, the meaning of them was not always that clear (*e.g.,* reverse causation, to be discussed in a latter part). Furthermore, a part of our data on serum BDNF concentrations showed a lack of fit with the expectations from the neurotrophin hypothesis. These non-confirmative findings are highlighted in the part that follows.

Serum BDNF concentrations - non-confirmative findings

A first finding that is not contingent on expectations is that depressed patients who were in the early remission phase of their depressive episode, and thus largely free of symptoms, had serum BDNF concentrations that were lower as compared to those of currently depressed patients (**chapter 4**). This finding does not relate well to the temporal dynamics specified in the neurotrophin hypothesis that low neurotrophic support should endanger a person to become depressed (*i.e.,* low BDNF → depression onset). In fact it suggests reversed causation, where lower serum BDNF concentrations are a consequence of depression (*i.e.,* depression → low BDNF).

Other findings reported in **chapter 4** and **5** that do not relate well to the neurotrophin hypothesis are that BDNF concentrations do not relate to the core clinical features of depression, such as its severity and that up-regulation of serum BDNF concentrations in the course of antidepressant treatment is confined to some classes of antidepressants. The finding on the lack of a relation between BDNF concentrations and clinical characteristics (notable depression symptom severity) does not confirm the logic of the linear dynamics of the neurotrophin hypothesis, from which it can be predicted that patients with higher symptom severity show particularly low neurotrophic support. Note that **chapter 5**, through meta-analysis, robustly confirms the absence of the association between serum BDNF concentrations and depression symptom severity, an association in which the literature had a great deal of belief. The finding of antidepressant specific effects on serum BDNF concentrations seems at odds with the prediction of the neurotrophin hypothesis that increases in BDNF concentrations are a key mediator for an antidepressant response to occur. Because, according to this prediction one might expect that antidepressants that are equally efficacious in the treatment of depression would have similar effects on serum BDNF concentrations, which they (according to our data but also that of others [Deuschle *et al.,* 2013]) obviously don't have. Another and related finding that is hard to reconcile with the neurotrophin hypothesis is that the group of depressed persons who used antidepressants (for a prolonged period and on a frequent base) had the highest serum BDNF concentrations alongside the highest symptom severity of depression (see **chapter 4**). This suggests that increases in peripheral BDNF concentrations occur in the course of treatment, but these do not parallel clinical effectiveness of antidepressants.

What **chapter 5** adds regarding the above-mentioned associations is that the literature is less reliable as

could be hoped for (*e.g.,* publication bias). In fact, and in contrast to prior data and belief, effect-sizes on between groups differences are only small and therefore the evidence for the neurotrophin hypothesis is less substantial as was initially thought. The small effect-size estimates further indicate that (changes in) serum BDNF concentrations probably are of little clinical use as a diagnostic- or treatment biomarker.

In **chapter 6** the expectation was that serum BDNF concentrations would be low in the anxious state. The data, however, gave little ground to this belief. In male patients with an anxiety disorder there were no abnormalities in serum BDNF concentration observed. In female patients with an anxiety disorder we found slightly lower serum BDNF concentrations as compared to healthy female. Therefore, the data in this chapter may attest that BDNF is involved in the pathophysiology of anxiety disorders *per se*. And, as explained (see the discussion on the relation between serum BDNF concentrations and estrogen), the alternations in female patients can easily be a female specific artifact of being anxious without being causally involved in anxiety etiology.

Finally, **chapter 7** attests the prevailing axiom that has been brought forward in explaining depression related alternations in BDNF expression: trauma exposure.

Clearly, findings that show a lack of fit with the expectations of the neurotrophin hypothesis are omnipresent in the studies that make up this thesis. Frankly, none of the expectations that can be derived from it could be fully validated. And if confirmative findings were reported, often their respective meaning was not that clear (*e.g.,* reverse causation). Maybe these non-confirmative findings by themselves are not a sufficient qualification to reject the neurotrophin hypothesis yet together they suggest that the initial idea of the neurotrophin hypothesis should not be credited.

The neurotrophin hypothesis: BDNF val^{66}met

In the **chapters 7** through **9** we explored associations between a variant on the gene that codes for BDNF, val^{66}met, for which functionality has been shown (*in vitro* and *in vivo*; in terms of neurotrophic support). We explicitly tested the expectation that the met-allele, the so called *risk allele* at this locus, is related to depression related phenotypes, with the latter being broadly defined from a DSM-IV depression diagnosis to impaired cognition and altered brain morphology. An important note is that trauma and stress exposure were taken into account in these studies in order to model inter-individual differences in outcomes due to these factors and their potential interaction with BDNF val^{66}met. Based on the presumed functionality of this polymorphism it was expected that the met allele would be associated with established correlates of depression, particularly in the face of trauma- and/or stress exposure.

BDNF val^{66}met - confirmative findings

A first confirmative finding (**chapter 7**) was a val^{66}met - trauma interaction effect on serum BDNF concentrations. This effect was such that BDNF met carriers had reduced serum BDNF concentrations but only when exposed to childhood abuse. In contrast to expectations was that this did not have any effect on behavior and the extent to which it is important thus remains to be elucidated. Furthermore, carriers of a met allele appeared to have somewhat lower hippocampal volumes relative to val/val homozygotes (**chapter 8**). Finally, hippocampal activity during the retrieval of negative words was different as a function of val^{66}met and trauma exposure (**chapter 8**). These findings may be in line with the expectation that the met-allele is a *risk allele* when depression related phenotypes are taken as outcome.

However, also with regard to val^{66}met this thesis reports findings that diverge from the predictions of the neurotrophin hypothesis. These inconsistencies are detailed below.

BDNF val^{66}met - non-confirmative findings

Findings that were not in line with a *priori* belief included that val^{66}met is not (directly) associated with psychopathology outcomes, cognitive performance, serum BDNF concentrations, or hippocampal activation patterns (**chapter 7** and **8**). Related is that the constellations of events/circumstances that had an effect on serum BDNF concentrations and hippocampal encoding activity (the met allele and exposure to childhood abuse) were not associated with expected outcomes at the behavioral level (**chapter 7** and **8**). Finally, and maybe most conclusive, **chapter 9** shows that that val^{66}met is not associated with hippocampal volume. This study in general is noteworthy in that it highlights the deleterious effects of underpowered studies and overestimations of effect-sizes that plague the field. Herewith the paper may be relevant beyond the val^{66}met – hippocampal volume literature. Given these non-confirmative findings I believe that val^{66}met is an invalid model to study BDNF functioning.

Conclusion

Confidence in a theory increases when it is confirmed by relevant data. Our data (and also that of others) show a lack of universal confirmation. Alongside some consistent findings, the data in the prevailing thesis largely detail inconsistencies regarding the neurotrophin hypothesis. And where expected associations were established (*e.g.,* abnormally low serum BDNF concentrations in the depressed state), the meaning often was not that clear (*e.g.,* reverse causation). I therefore conclude, whilst taking limitations into account and acknowledging that the results are contingent upon imperfect measurement that the most reliable evidence in humans does not corroborate the neurotrophin hypothesis. So, given the data, the final words of this thesis are that solid work over novelty shows that the neurotrophin hypothesis should no longer be credited in its original form. *All that glitters is not gold* - back to the drawing table.

Future work – the drawing table

At the drawing table I could come up with some aspirational goals. The one of these with greatest importance is to deepen the understanding of how neurotrophic functioning may be altered in the depressed state (*i.e.,* construct validity) and the accompanying functional consequences of this on health (*i.e.,* predictive validity). Alongside this, the temporal dynamics as specified in the neurotrophin hypothesis (*i.e.,* low BDNF support → depression onset) should be entangled because now reversed causation (*i.e.,* depression onset → low BDNF support) in which low BDNF support does not mark the beginning of a depressive episode but rather a consequence of it can not be excluded (*i.e.,* construct validity). Further challenges on the way to progress include collaboration in data-sharing networks to answer, with rigor, some outstanding questions (*i.e.,* statistical validity), to measure beyond single BDNF parameters, to bring the preclinical and clinical research more together, and, in parallel, to leave behind the diagnostic categories of the DSM in the study of neurotrophic functioning (*i.e.,* all construct validity). Once this can be established, progress can be made and the question -- *will the neurotrophin hypotehsis sparkle on, long after the glitter of the firework is gone* -- can definitely be dealt with, maybe in the end accompanied by hitherto useful information for clinical areas.

NEDERLANDSE SAMENVATTING EN CONCLUSIES

De rol van BDNF in depressie *-- Zal de neurotrofe hypothese nog lang schitteren?*

ACHTERGROND

Neuronale plasticiteit is van essentieel belang voor het functioneren van basale processen als het geheugen, leervermogen en emotie regulatie. Het neuronale groeihormoon Brain-Derived Neurotrophic Factor (BDNF) induceert en medieert neuronale plasticiteit. Men vermoedt dat inzichten in het ontstaan en het verloop van verschillende psychiatrische aandoeningen vergroot kunnen worden door de regulatie van BDNF beter te begrijpen.

Volgens de neurotrofe hypothese is een laag niveau van BDNF het startpunt van een pathophysiologische cascade, die via verstoorde neuronale plasticiteit en atrofie in hersengebieden die emotie reguleren, kan leiden tot een depressieve stoornis. Er is toenemend preklinische bewijs voor deze hypothese en er wordt veel ondezoek verricht om tot een vertaalslag naar de menselijke depressie te komen. Zo toont een aantal humane studies aan dat perifere parameters voor neurotroof functioneren (in de vorm van bijvoorbeeld serum BDNF-spiegels) anders worden gereguleerd bij mensen die lijden aan een depressieve stoornis. Ook laten enkele studies zien dat genetische varianten, waarvan preklinisch werk heeft aangetoond dat ze de expressie van BDNF reguleren, associaties vertonen met aan depressie gerelateerde fenotypen.

Ondanks positieve geluiden over de neurotrofe hypothese en de mogelijke implicaties die dit model zou kunnen bieden om depressieve stoornissen beter te begrijpen, is er ook onzekerheid. De voornaamste bron van onzekerheid komt voort uit een gebrek aan kennis over de fundamentele determinanten van serum BDNF-spiegels. Gebrek aan kennis op dit gebied kan immers eenvoudig leiden tot onterechte conclusies uit het voornamelijk observationele onderzoek dat tot dusver is uitgevoerd naar de rol van BDNF in depressie. Een tweede bron van onzekerheid is dat een reeks klinische vragen vooralsnog onbeantwoord blijft. Met dit proefschrift probeer ik antwoord te geven op enkele vragen met betrekking tot de neurotrofe hypothese en zodoende een verfijnd beeld van perifeer neurotroof functioneren in depressieve (en verwante) stoornissen te schetsen. Belangrijk is hierbij de methode van aanpak. In tegenstelling tot het gros van het eerdere onderzoek op deze gebieden wordt gebruikgemaakt van grote groepen en meta-analyses om zo associaties betrouwbaar te onderbouwen dan wel te weerleggen.

Acht empirische studies vormen het hart van dit proefschrift. In de paragraaf die volgt vat ik de resultaten van deze studies samen (per hoofdstuk) en geef ik kort het belang aan van de desbetreffende studies. Het eerste doel van dit proefschrift, het blootleggen van de determinanten van serum BDNF spiegels, wordt beschreven in de **hoofdstukken 2** en **3**. De **hoofdstukken 4** - **9** staan in het teken van klinische vragen met betrekking tot de neurotrofe hypothese.

RESULTATEN

Het eerste empirische hoofdstuk van dit proefschrift **(hoofdstuk 2)** geeft een gedetailleerde beschrijving van de determinanten van serum BDNF spiegels. De studie toont aan dat een niet-nuchtere toestand op het moment van bloedafname, een later tijdstip van bloedafname en een langere duur van serum opslag gepaard gaan met verlaagde serum BDNF-spiegels. Dit in tegenstelling tot roken en leven in een stedelijk gebied (in vergelijking tot een meer landelijk gebied), die beide worden geassocieerd met verhoogde BDNF-spiegels. Tenslotte lijken serum BDNF-spiegels toe te nemen als een functie van leeftijd.

Deze paper schetst de determinanten van serum BDNF-spiegels. Dit is een belangrijk uitgangspunt om interindividuele verschillen in serum BDNF-spiegels te kunnen begrijpen en is van essentieel belang om tot

valide conclusies te komen in onderzoek naar BDNF.

In **hoofdstuk 3** is onderzocht of serum BDNF-spiegels seizoensgebonden zijn. Hiertoe werden in eerste instantie analyses uitgevoerd om te bepalen of BDNF-spiegels verschilden als een functie van de maand waarin het bloed was afgenomen om BDNF in te bepalen. De resultaten tonen uitgesproken seizoensgebonden variatie: serum BDNF-spiegels nemen toe in de lente/zomer periode (*i.e.,* de lente equinox) en af in de loop van de herfst/winter periode (*i.e.,* de herfst equinox). Verder bleek dat de lengte van de dag en het aantal uren zonneschijn, twee zogeheten *zeitgebers* (triggers van seizoensgebonden variatie) in de weken voor bloedafname positief gecorreleerd zijn aan serum BDNF-spiegels.

De bevindingen van deze studie zijn van meerwaarde, omdat ze inzicht verschaffen in de factoren die BDNF- expressie reguleren. Daarnaast zijn ze van cruciaal belang bij het ontwerpen en evalueren van studies die BDNF spiegels als uitkomst? variabele meenemen.

Het **4de hoofdstuk** van dit proefschrift gaat in detail in op het verband tussen serum BDNF-spiegels en depressie. De belangrijkste bevinding in dit hoofdstuk is dat serum BDNF-spiegels laag zijn bij depressieve patiënten die niet worden behandeld met antidepressiva ten opzichte van gezonde controles en van depressieve patiënten die wel worden behandeld met een antidepressivum. Daarnaast laat dit hoofdstuk zien dat serum BDNF-spiegels van mensen met een depressie in de remissie-fase vergelijkbaar zijn met die van gezonde controles. Verder bleek dat serum BDNF-spiegels niet zijn gerelateerd aan de klinische kenmerken van depressie, zoals de ernst van de stoornis.

Deze studie laat zien dat lage serum BDNF-spiegels een 'state' karakteristiek is van de depressieve stoornis, een abnormaliteit die aanwezig is tijdens de daadwerkelijke depressieve episode en die normaliseert in de loop van natuurlijke remissie en behandeling met antidepressiva. Van kritisch belang is echter dat deze studie ook laat zien dat normalisatie van serum BDNF-spiegels niet noodzakelijkerwijs geassocieerd is met een verlichting van de depressieve symptomen.

Hoofdstuk 5 rapporteert de bevindingen van een aantal meta-analyses over verschillen in serum BDNF-spiegels tussen depressieve patiënten en gezonde controles. De analyses laten zien dat serum BDNF-spiegels laag zijn bij depressieve patiënten die niet worden behandeld met een antidepressivum in vergelijking tot gezonde controles, en depressieve patiënten die wel waren behandeld met een antidepressivum. Deze laatste groep verschilt niet van gezonde controles wat betreft serum BDNF-spiegels. Verder is er grote heterogeniteit in uitkomsten tussen studies, waar geen theoretisch relevante verklaring (*e.g.,* ernst van de depressie) voor kon worden gevonden. Wel was er evidentie voor enkele 'artificiële' verklaringen. Zo rapporteren minder betrouwbare studies relatief grote effect-groottes en is er sterk bewijs voor publicatie bias. Indien voor dit laatste een statistische correctie werd toegepast dan worden de effect-groottes over de associaties substantieel kleiner, maar blijven significant. Voor de relatie tussen de ernst van de depressie en serum BDNF spiegels geeft de gepoolde data geen consistent bewijs.

Deze studie is opmerkelijk, niet zozeer omdat wordt bevestigd dat veranderingen in serum BDNF-spiegels perifere manifestaties van depressie lijken te zijn, maar eerder doordat het laat zien dat het bewijs voor dit idee niet zo sterk is als aanvankelijk werd gedacht. Een belangrijke implicatie van de boodschap in dit hoofdstuk is dat kennis met betrekking tot serum BDNF-spiegels waarschijnlijk weinig (directe) klinische relevantie heeft.

Hoofdstuk 6 vergelijkt serum BDNF-spiegels tussen patiënten met een angststoornis en gezonde controles. Angststoornissen overlappen op een groot aantal dimensies met depressie en hierdoor was de verwachting

dat serum BDNF-spiegels abnormaal laag zouden zijn bij patiënten met een angststoornis. Over het geheel genomen ondersteunen de bevindingen van deze studie deze verwachting niet, want serum BDNF-spiegels lijken vergelijkbaar tussen patiënten en controles. Wel hadden vrouwelijke patiënten met een angststoornis lagere serum BDNF-concentraties ten opzichte van gezonde vrouwen, waar dit voor mannen niet het geval is. Een laatste bevinding uit deze studie is dat serum BDNF-spiegels niet zijn gerelateerd aan de klinische karakteristieken van angst, bijvoorbeeld de duur van de symptomen.

De resultaten van deze studie bevestigen niet de verwachting dat serum BDNF spiegels laag zijn in mensen met een angststoornis. De studie laat wel iets lagere serum BDNF-spiegels zien bij vrouwelijke patiënten met een angststoornis. Deze bevinding kan erop wijzen dat BDNF een rol speelt in de pathologie van angst bij vrouwen of, niet onwaarschijnlijk, een vrouwelijke specifiek artefact van angst weerspiegelt.

Hoofdstuk 7 toetst de hypothese dat het BDNF val^{66}met genotype gerelateerd is aan serum BDNF spiegels. De studie vindt geen direct bewijs voor deze hypothese, maar wel dat dragers van het met allel lagere BDNF spiegels hebben in vergelijking tot mensen die homozygoot zijn voor het val allel wanneer zij zijn blootgesteld aan kindermishandeling. Bovendien hebben dragers van het met allel hogere BDNF-spiegels dan mensen die homozygoot zijn voor het val allel, wanneer ze zijn niet blootgesteld aan kindermishandeling. Daarnaast bevestigt deze studie de neurotrofe hypothese in zijn voorspelling dat blootstelling aan recente stressvolle gebeurtenissen wordt geassocieerd met een (kleine) afname in serum BDNF-spiegels.

Blootstelling aan stress en/of trauma is volgens de neurotrofe hypothese het axioma dat de lagere BDNF-spiegels verklaardt in mensen met aan stress gerelateerde psychopathologie. Het belang van dit paper is dat het dit idee niet weet te bevestigen. Wel laat deze studie een interessant interactie effect zien tussen val^{66}met en kindermishandeling. De mate waarin deze interactie belangrijk is (op verschillende niveaus van functioneren) dient in vervolgonderzoek te worden bekeken.

In **hoofdstuk 8** is onderzocht of het volume en de functionaliteit van de hippocampus zijn gerelateerd aan BDNF val^{66}met. Hiertoe is gebruikgemaakt van structurele en functionele MRI-data. De resultaten van dit onderzoek wijzen uit dat dragers van een BDNF met allel kleinere hippocampi hebben ten opzichte val/val homozygoten. Verder rapporteert deze studie dat dragers van een met allel verhoogde neurale activiteit vertonen in de hippocampus in hun reactie op emotionele beladen woorden. Consistente associaties tussen val^{66}met en cognitief functioneren en psychopathologie zijn niet evident in deze studie.

De boodschap in dit hoofdstuk is van belang omdat deze het begrip van de veelbesproken associaties tussen BDNF val^{66}met met neuronaal functioneren uitdiept. Deze studie, hoewel de bevindingen onafhankelijk gerepliceerd dienen te worden, suggereert dat dragers van een met allel abnormale reacties vertonen in reactie op emotioneel geladen stimuli. Hierbij dient wel te worden opgemerkt dat de gedragseffecten (op bijvoorbeeld cognitief functioneren of psychopathologie) die men zou verwachten naast de neurobiologische verschillen niet zijn waargenomen.

Hoofdstuk 9 presenteert een meta-analyse over de relatie tussen BDNF val^{66}met en het volume van de hippocampus. De resultaten van deze analyse suggereren dat de hippocampus van dragers van een met allel kleiner is qua omvang in vergelijking tot die van val/val homozygoten. Echter, de heterogeniteit in uitkomsten tussen studies is aanzienlijk, wat niet kan worden verklaard door demografische, klinische en methodologische karakteristieken van de geïncludeerde studies. Wel zijn er sterke aanwijzingen voor publicatie-bias en effect-groottes nemen af als een functie van jaar van publicatie. Dit alles duidt erop dat

kleinere hippocampi waarschijnlijk niet een biologisch effect van het met allel zijn maar eerder een methodologisch artefact.

De bevinding dat dragers van een met allel, wat volgens preklinische studies wordt geassocieerd met lagere neurotrofe ondersteuning, kleinere hippocampale volumes hebben is in de loop der jaren een pijler voor de neurotrofe hypothese geworden (het totale aantal citaties naar de eerste paper over deze relatie [Pezawas *et al.,* 2005] is > 500). Deze studie toont echter aan dat dit verband waarschijnlijk een artefact is: gebaseerd op studies met een te lage statistische power.

METHODEN

Voordat ik de belangrijkste discussiepunten en de conclusies van dit proefschrift aan u presenteer, vat ik eerst de sterke- en zwakke punten van de studies in dit proefschrift kort samen. Dit is namelijk van groot belang bij de interpretatie van de resultaten in dit proefschrift en de manier waarop ik daar betekenis aan heb gegeven.

Sterk is dat bijna alle resultaten in dit proefschrift gebaseerd zijn op een grote steekproef, wat betrouwbare schattingen van effect-groottes biedt (Big Data Notable Fidelity). Een ander sterk punt is dat de resultaten in dit proefschrift een hoge mate van validiteit hebben doordat er statistisch is gecontroleerd op verstorende factoren, en dat er moderatie- en subgroep analyses zijn uitgevoerd. Daarnaast is in een aantal gevallen de betrouwbaarheid van de onderzoeksresultaten getoetst door middel van meta-analyse.

Naast deze sterke punten kennen de studies in dit proefschrift ook de nodige beperkingen. Allereerst zijn onze conclusies grotendeels afhankelijk van perifere maten (d.w.z. serum BDNF) waar neurotroof functioneren een centraal proces betreft. Ten tweede, de resultaten in dit proefschrift zijn gebaseerd op voornamelijk cross-sectioneel onderzoek en in geen van de studies is gebruik gemaakt van randomisatie. De gerapporteerde bevindingen zijn hierdoor niet voldoende overtuigend om causaliteit aan te tonen. Tot slot, de resultaten die worden gerapporteerd in dit proefschrift zijn niet direct te generaliseren naar alle populaties, met name niet naar kinderen/adolescenten en mensen die aan zeer ernstige psychopathologie lijden.

DISCUSSIE

Dit proefschrift voegt twee belangrijke elementen aan de wetenschappelijke literatuur toe. Ten eerste biedt het een overzicht van de determinanten van serum BDNF-spiegels. Ten tweede levert het antwoorden op een aantal belangrijke klinische vragen die voortkomen uit de neurotrofe hypothese. Dit tezamen heeft aanzienlijke methodologische en theoretische implicaties die hieronder zullen worden samengevat. Ik begin met de bevindingen betreffende de determinanten van serum BDNF-spiegels.

Deel I: determinanten

Inzicht in de determinanten van een variabele is van vitaal belang om tot valide conclusies te komen. De eerste twee hoofdstukken van dit proefschrift beschrijven de resultaten van onze pogingen om inzicht te krijgen in de determinanten van serum BDNF-spiegels.

Dit proefschrift beschrijft een aantal variabelen die op systematische wijze samenhangen met serum BDNF-spiegels, waaronde enkele die, wanneer niet op adequate wijze onder controle, gemakkelijk kunnen leiden tot niet valide conclusies in het testen van de voorspellingen van de neurotrofe hypothese. Een voorbeeld dat dit goed illustreert is het seizoensgebonden effect in serum BDNF-spiegels waar wij over rapporteren in **hoofdstuk 3**. Het is een gegeven dat de prevalentie van depressie hoger is in de winter in vergelijking tot de zomer. De sterke seizoen gebondenheid in serum BDNF-spiegels (**hoofdstuk 3**), samen met de hogere prevalentie cijfers van depressie in de winter, suggereert dat het controleren voor seizoen

van belang is wanneer men bijvoorbeeld het verschil in serum BDNF spiegels tussen depressieve patiënten en gezonde controles wil bepalen. Dit blijkt inderdaad zo te zijn, daar de effect-grootte over deze associatie met 40 procent afneemt in analyses die zijn gecontroleerd voor het seizoen waarin de deelnemers zijn gesampled.

Zoals beschreven heeft onze groep vooruitgang weten te boeken in het begrip van de determinanten van serum BDNF-spiegels. De focus lag hierbij op methodologische aspecten, dat wil zeggen: hoe kunnen de relaties met onze primaire interesse (*i.e.,* vragen die voortvloeien uit de neurotrofe hypothese) zo zuiver mogelijk worden getoetst. De bevindingen die worden gerapporteerd in **hoofdstuk 2** en **3** nodigen uit tot een perspectief op BDNF gerelateerd onderzoek waarin de basale determinanten zijn geïntegreerd/gecontroleerd.

Deel II: de neurotrofe hypothese

In de **hoofdstukken 4** - **7** onderzochten we serum BDNF-spiegels in relatie tot psychiatrische ziektebeelden, met name depressie, de karakteristieken en de behandeling ervan. Expliciet getest zijn de volgende (vermeende) associaties: (**I**) patiënten met een depressieve stoornis (en verwante aandoeningen) vertonen consistent lage serum BDNF-spiegels, (**II**) serum BDNF-spiegels zijn gerelateerd aan kenmerken van de depressieve stoornis (met name de ernst van de symptomen) en (**III**) gebruik van antidepressiva en risicofactoren voor het ontwikkelen van een depressie (*i.e.,* blootstelling aan trauma en stress) zijn gerelateerd aan serum BDNF-spiegels. Een aantal van deze verwachtingen wordt bevestigd in dit proefschrift, en vormen het onderwerp van de alinea die volgt.

Bevestigde verwachtingen: de neurotrofe hypothese - serum BDNF-spiegels

In lijn met de *a priori* verwachtingen rapporteren we in **hoofdstuk 4** en **5** dat serum BDNF-spiegels significant verlaagd zijn bij depressieve patiënten die niet worden behandeld met een antidepressivum ten opzichte van gezonde controles en depressieve patiënten die wel worden behandeld met een antidepressivum. De resultaten in **hoofdstuk 4** en **5** geven verder aan dat serum BDNF-spiegels normaliseren in de loop van volledige remissie. De conclusie die hieruit volgt is dat lage serum BDNF-spiegels een 'state' kenmerk zijn van depressie: laag tijdens de actieve fase van de stoornis en genormaliseerd wanneer de depressie in volledige remissie is.

In overeenstemming met de verwachtingen van de neurotrophe hypothes, lijken serum BDNF-spiegels zich in de loop van de behandeling met antidepressiva, te normaliseren, en worden vergelijkbaar aan de waarden van die van gezonde controles. Een belangrijke noot hier is dat de hierboven genoemde bevindingen robuust zijn: afgeleid van de grootste (en meest betrouwbare) enkelvoudige studie (**hoofdstuk 4**) en bevestigd door meta-analyse (**hoofdstuk 5**).

Hoofdstuk 6, kan gezien worden als een extensie van de neurotrofe hypothese naar de angststoornissen, aangezien we daar vinden wij dat vrouwelijke patiënten met een angststoornis lage BDNF-spiegels hebben ten opzichte van gezonde vrouwen. Dit zou kunnen betekenen dat BDNF een rol speelt in de pathofysiologie van angst bij vrouwen.

Tenslotte, en mede in lijn der verwachting van de neurotrofe hypothesis is de bevinding in **hoofdstuk 7**, dat blootstelling aan recente stressvolle gebeurtenissen (doorgaans etiologische risicofactoren voor de ontwikkeling van een depressie), geassocieerd blijkt te zijn met een (kleine) daling in serum BDNF-spiegels.

Ondanks dat de bovengenoemde bevindingen als bevestigingen van de neurotrofe hypothese worden gezien, moet worden erkend dat hun betekenis niet altijd even duidelijk is (omgekeerde causaliteit, bijvoorbeeld, kan niet worden uitgesloten [dit wordt in een later deel van deze samenvatting besproken]). Daarnaast rapporteren de studies in dit proefschrift een scala aan bevindingen die niet in de pas lopen met

de voorspellingen van de neurotrofe hypothese of die hier zelfs haaks op staan. Deze worden kort bediscussieerd in de paragraaf die volgt.

Serum BDNF-spiegels – niet bevestigende verwachtingen

Een eerste bevinding die duidelijk niet in lijn is met de verwachtingen van de neurotrofe hypothese is dat depressieve patiënten die in de vroege remissiefase van hun depressieve episode zitten, en dus grotendeels vrij van symptomen zijn, serum BDNF-spiegels hebben die lager zijn dan die van huidig depressieve patiënten (**hoofdstuk 4**). Deze bevinding past niet goed binnen de temporele dynamiek die de neurotrofe hypothese specificeert: dat lage BDNF-spiegels, of neurotrofe ondersteuning in het algemeen, een persoon kwetsbaar maken om depressief te worden (*d.w.z.*: laag BDNF → verhoogde kans op depressie). In feite suggereert deze bevinding omgekeerde causaliteit, dat lage serum BDNF-spiegels het gevolg zijn van de depressieve episode (*d.w.z.*: depressie → verlaging van BDNF).

Een andere bevindingen die wordt gerapporteerd in **hoofdstuk 4** en **5** die niet rijmt met de neurotrofe hypothese is de afwezigheid van consistente associaties tussen serum BDNF-spiegels en de klinische kenmerken van depressie. Met name het ontbreken van een relatie tussen serum BDNF-spiegels en de ernst van de depressie, druist in tegen de logica van de lineaire dynamiek van de neurotrofe hypothese, waaruit kan worden afgeleid dat patiënten met een hogere mate van ernst ook een lagere neurotrofe ondersteuning hebben. In **hoofdstuk 5**, waarin een meta-analyse over deze associatie wordt gerapporteerd, wordt de afwezigheid van deze vermeende associatie robuust bevestigd. Dit is een belangrijke bevinding, aangezien het geloof in het bestaan van deze relatie groot was.

Daarnaast rapporteert **hoofdstuk 4**, evenmin in lijn met de neurotrofe hypothese, dat de normalisatie van BDNF-spiegels in de loop van de behandeling met antidepressiva beperkt lijkt tot sommige klassen van antidepressiva. Deze bevinding staat op gespannen voet met de voorspelling van de neurotrofe hypothese dat een toename in de expressie van BDNF de mediator is voor de klinische response op antidepressiva. Deze voorspelling volgend zou men namelijk verwachten dat antidepressiva die klinisch gezien ongeveer even effectief zijn in de behandeling van depressie ook gelijke associaties zouden vertonen met serum BDNF-spiegels. En deze gelijke associatie hebben zij volgens onze data (**hoofdstuk 4**), maar ook die van anderen, duidelijk niet. Een gerelateerde bevinding die moeilijk is te rijmen met de neurotrofe hypothese is dat de groep van depressieve personen die antidepressiva gebruikt (voor een langere periode en op een frequente basis) de hoogste serum BDNF-spiegels hebben maar ook gemiddeld de hoogste ernst van depressie (**hoofdstuk 4**). Dit suggereert dat de verhogingen of normalisatie van serum BDNF-spiegels die worden geobserveerd in de loop van de behandeling met antidepressiva niet parallel lopen met de klinische effectiviteit van het medicijn.

Een belangrijk inzicht van **hoofdstuk 5** is dat de literatuur over de eerder genoemde associaties minder betrouwbaar is dan kan worden gehoopt. De redenen hiervoor zijn onder meer publicatie bias. In feite, een belangrijke les die **hoofdstuk 5** ons brengt is dat in tegenstelling tot wat eerder werd gedacht, dat de effect – groottes voor de groepsverschillen met onze interesse slechts klein zijn of zelfs afwezig. Dit impliceert dat serum BDNF-spiegels waarschijnlijk niet van enig nut zijn in een rol als diagnostische- of behandeling 'biomarker'.

De *a priori* verwachting in **hoofdstuk 6**, gebaseerd op de neurotrofe hypothese, was dat serum BDNF-spiegels laag zouden zijn bij mensen die zijn gediagnosticeerd met een angststoornis. De data in dit hoofdstuk geeft echter weinig onderbouwing voor deze verwachting, omdat er in het algemeen geen verschillen werden gevonden in serum BDNF-spiegels tussen mensen met een angststoornis en gezonde controles. Hieruit volgt de conclusie dat BDNF waarschijnlijk niet een rol van betekenis speelt in de pathofysiologie van angststoornissen *per se*. Zoals reeds genoemd vonden we bij vrouwelijke patiënten met

een angststoornis enigszins lagere serum BDNF spiegels in vergelijking met gezonde vrouwen. Dit zou kunnen wijzen op een specifieke rol van BDNF in de etiologie van angststoornissen bij vrouwen, maar deze abnormaliteit kan evengoed een vrouwelijke specifiek artefact van een angststoornis representeren.

In **hoofdstuk 7** hebben we het in de neurotrofe hypothese heersende axioma getest dat blootstelling aan stress en trauma de lagere BDNF spiegels in aan stress gerelateerde stoornissen verklaart. Ook voor dit axioma vinden wij geen consistent bewijs.

Kortom, bevindingen die niet aansluiten bij de verwachtingen van de neurotrofe hypothese zijn alomtegenwoordig in de studies in dit proefschrift. In feite kan geen van de verwachtingen van de neurotrofe hypothese volledig worden gevalideerd. En daarnaast, als onze studies enigszins bevestigende resultaten opleverden dan was hun betekenis vaak niet duidelijk (omgekeerde causaliteit bijvoorbeeld). Misschien zijn deze niet- bevestigende bevindingen ieder voor zich niet voldoende om de neurotrofe hypothese als niet-valide te bestempelen. Samen suggereren zij echter dat het attribueren van gedragsverschillen (zoals depressie) aan verschillen in serum BDNF-spiegels een stap te ver gaat. Daarom zal de neurotrofe hypothese in zijn oorspronkelijke vorm niet behouden kunnen worden.

Deel II vervolg: de neurotrofe hypothese - BDNF val^{66}met

De **hoofdstukken 7**, **8** en **9** verkenden associaties tussen een variant op het gen dat codeert voor BDNF: val^{66}met. Deze variant is in preklinisch werk (*in vitro* en *in vivo*) functioneel gebleken in termen van de expressie van BDNF. Uitdrukkelijk hebben wij de verwachting getoetst dat het met allel, het risico allel op deze locus, is gerelateerd aan depressie gerelateerde fenotypen, waaronder de DSM-IV depressie diagnose, cognitief functioneren en hippocampale morfologie en functionaliteit. Belangrijk is dat de effecten van blootstelling aan trauma en stress ook zijn gemodelleerd in deze studies, naast hun potentiële interactie effecten met BDNF val^{66}met. De verwachting betreffende dit laatste effect was dat dragers van een met allel met name in aanwezigheid van trauma en/of stress blootstelling slecht af zouden zijn betreffende de aan depressie gerelateerde fenotypen die wij als uitkomstmaat gekozen hebben (*e.g.,* de ernst van de symptomen).

Bevestigende verwachtingen: de neurotrofe hypothese - BDNF val^{66}met

Een eerste bevinding in **hoofdstuk 7** die de neurotrofe hypothese in zekere mate lijkt te bevestigen is een val^{66}met - trauma interactie-effect op de serum BDNF-spiegels. Dit effect is zodanig dat dragers van een met allel lagere BDNF-spiegels hebben in vergelijking tot val/val homozygoten, maar alleen wanneer zij zijn blootgesteld aan trauma in de jeugd. In tegenstelling tot wat was verwacht hadden met drager die aangaven getraumatiseerd te zijn geen ander klinisch profiel (*bijvoorbeeld* de ernst van de depressie was vergelijkbaar). De klinische relevantie van dit interactie effect blijft dus vooralsnog onduidelijk.

Naast deze intrigerende bevinding rapporteren wij in **hoofdstuk 8** lagere hippocampale volumes in dragers van een met allel ten opzichte val/val homozygoten. Tenslotte vonden wij in dit hoofdstuk ook dat hippocampale activiteit tijdens het ophalen van negatieve woorden uit het geheugen hoger is in dragers van een met allel relatief tot dat van val/val homozygoten. Deze bevindingen lijken in overeenstemming met de gedachte dat het met allel een risico allel is waar het gaat om aan depressie gerelateerde fenotypes.

Echter, ook met betrekking tot het val^{66}met polymorfisme worden in dit proefschrift bevindingen gerapporteerd die afwijken van de voorspellingen door de neurotrofe hypothese. Deze inconsistenties worden in de alinea hieronder beschreven.

Niet bevestigende verwachtingen: de neurotrofe hypothese - BDNF val^{66}met

Inconsistent met de neurotrofe hypothese zijn de bevindingen in **hoofdstuk 7** en **8** dat val^{66}met niet (direct) is gerelateerd aan psychopathologie, cognitief functioneren, serum BDNF-spiegels, noch aan hippocampale activiteit. Gerelateerd is dat de constellaties van gebeurtenissen of omstandigheden die een effect op serum BDNF-spiegels en hippocampale coderings-activiteit hadden (dat is, het met allel plus blootstelling aan trauma) geen effect hadden op uitkomsten op gedragsniveau (**hoofdstuk 7** en **8**). Ten slotte, en misschien wel het meest overtuigend, suggereert de meta-analyse in **hoofdstuk 9** dat val^{66}met niet is geassocieerd met het volume van de hippocampus. De studie in **hoofdstuk 9** is verder opmerkelijk omdat het wijst op de verstorende effecten van kleine steekproeven en overschatte effect-groottes. Hiermee is de boodschap van dit paper ook relevant voor hersenonderzoek naar de impact van andere kandidaat genen, ook buiten het val^{66}met onderzoeksveld om.

Gezien deze bevindingen, en andere recente en betrouwbare literatuur concludeer ik dan ook dat het bewijs voor BDNF val^{66}met als model voor neurotroof functioneren tanende is.

Conclusie

Bevestigende onderzoeksbevindingen scheppen vertrouwen in een theorie of hypothese. Dit proefschrift brengt, naast enkele resultaten die consistent lijken te zijn met de neurotrofe hypothese, grotendeels inconsistenties betreffende het raamwerk van voorspellingen die deze hypothese naar voren brengt. Waar wel consistente bevindingen zijn gevonden (bijvoorbeeld abnormaal lage serum BDNF-spiegels in de depressieve toestand), is de betekenis vaak niet duidelijk (bijvoorbeeld omgekeerde causaliteit). Ik concludeer derhalve, terwijl ik de beperkingen van het onderzoek in acht neem, dat de neurotrofe hypothese in zijn oorspronkelijke vorm niet als valide mag worden bestempeld – er zijn simpelweg teveel inconsistenties.

Toekomstig werk

Op een aantal fronten zijn doelstellingen te formuleren die kunnen leiden tot vooruitgang in het onderzoeksveld waar ik de afgelopen jaren in actief ben geweest. De doelstelling die naar mijn mening van het grootste belang is, is om beter te begrijpen wat de lage BDNF-spiegels veroorzaakt bij depressieve mensen (construct validiteit) en wat de functionele gevolgen hiervan zijn voor de gezondheid (predictieve validiteit). Daarnaast zal de temporele dynamiek zoals die is gespecificeerd in de neurotrofe hypothese grondig onderzocht moeten worden. De stand van zaken is nu zo dat omgekeerde causaliteit waarin lage neuronale ondersteuning door BDNF niet het begin is van een depressieve episode, maar een gevolg ervan (construct validiteit) niet uitgesloten kan worden. Verder zal de aandacht gericht moeten worden op grootschalige samenwerkingsverbanden en data-sharing om op solide wijze antwoord te kunnen geven op een aantal openstaande vragen (statistische en construct validiteit). Andere uitdagingen zijn onder meer: (**I**) onderzoek waarin meerdere parameters voor neurotroof functioneren en voor gerelateerde biologische processen worden gemeten, (**II**) om het preklinische en klinische onderzoek meer bij elkaar te brengen en (**III**) de brede diagnostische categorieën in de studie van neurotroof functioneren te verlaten (construct validiteit). Door dit alles kan vooruitgang worden geboekt en zal de vraag of de neurotrofe hypothese nog lang zal schitteren misschien kunnen worden beantwoord, wellicht vergezeld van nuttige informatie voor de klinische praktijk.

DEUTSCHE ZUSAMMENFASSUNG UND SCHLUSSFOLGERUNGEN

Die Rolle von BDNF in depressive Störungen

HINTERGRUND

Wo angenommen wird, sind Neurotrophe Faktoren sehr wichtig für das normale Funktionieren der neuronalen Plastizität, fur Gedächtnis, Lernen und fur Stimmung. Die Neurotrophine Brain-Derived Neurotrophic Factor (BDNF) ist der wichtigste Vermittler von neurotrofe Unterstützung und es wurde erklärt, dass durch die Regulierung von BDNF kennen zu lernen das Verständnis von verschiedenen psychiatrischen Erkrankungen erhöht werden kann. Nach der Neurotrofe Hypothese entstehen depressive Störungen durch abweichende neurotrophe Unterstützung durch BDNF in Hirnregione die Emotionen regulieren. Im Laufe der Jahre hat sich diese Hypothese an stabilen Beweise gewonnen. Darüber gibt es Grund zu der Überzeugung, dass periphere Maßnahmen (*z. B.* BDNF-Spiegel im Blut) und bestimmte genetische Varianten (*z. B.* BDNF val^{66}met) als Fenster für neurotrophic Aktion im Gehirn funktionieren kann.

Allerdings, inmitten einer Menge Aufregung besteht Unsicherheit über die Vorhersagen der Neurotrophin Hypothese. Quellen der Unsicherheit betrachten einen Mangel an Wissen über die Determinanten von Serum BDNF-Spiegel und wichtige klinische Fragen die bislang unbeantwortet sind. In dieser Arbeit richtete ich mich genau auf diese Punkten, um so ein verfeinertes Modell des (peripheren) neurotrophischen funktionieren in depressiven (und verwandte) Erkrankungen zu bieten. Eine Kraft von der aktuellen Arbeit ist, dass sie is basiert auf große Einzelstudien und Meta-analytische Techniken.

Die empirischen Daten, die die Grundlage von dieser Arbeit machen, und eine Diskussion über sie, sind in den vorangegangenen Kapiteln dargestellt. Eine Zusammenfassung der Studien und der Hauptpunkte der Diskussion wird im folgenden Abschnitt vorgestellt.

ERGEBNISSE

Im Folgenden werden die Ergebnisse unserer empirischen Studien präsentiert (nach Kapitel) neben der Bedeutung, die ich glaube dass sie möglicherweise hatten. Das erste Ziel dieser Arbeit, das abzugrenzen der Serum BDNF-Spiegel Determinanten, wird in **Kapitel 2** und **3** angegeben. Die **Kapitel 4** bis **9** beantworten die klinischen Fragen.

TEIL I: Determinanten

Kapitel 2 bietet eine detaillierte Beschreibung der Determinanten von Serum BDNF-Spiegeln. Es zeigt , bei Personen die unbehandelt mit Antidepressiva und frei von einer aktuellen psychiatrischen Krankheit sind, dass eine nicht nüchternen Zustand zum Zeitpunkt der Blutprobe, spätere Blutprobe am Tag, eine längere Lagerzeit von Blutserum und *Binge* Trinken alle im Zusammenhang mit abgeschwächten Serum BDNF-Spiegels sind. Dies im Gegensatz zum Rauchen und das Leben in einem städtischen Gebiet, das mit einem erhöhten BDNF-Spiegel verbunden war. Schließlich hatten ältere Probanden höhere Serum BDNF-Spiegels, aber dies erwies sich besonders für Frauen (*i.e.,* eine Geschlecht-Alter Interaktionseffekt).

Der Hauptgedanke dieser Arbeit ist, dass sie die Determinanten von Serum BDNF-Spiegels skizziert. Hiermit bietet sie eine verbesserte Basis, um interindividuelle Unterschiede im Serum BDNF-Spiegels zu verstehen. Darüber hinaus sind diese Daten sehr wichtig, um zu gültige Schlussfolgerungen zu gelangen in der BDNF-Forschung.

In **Kapitel 3** untersuchten wir saisonalen Regulierung von Serum BDNF-Spiegel. Analysen nach Monat der Probenahme zeigten ausgeprägte saisonalen Schwankungen. BDNF-Spiegels im Serum erhöhten sich über den Frühjahr-Sommer Zeitraum und verringerte sich über den Herbst-Winter Zeitraum. Die natürliche Tageslänge und die Anzahl der Sonnenstunden (wichtigsten Auslöser der Saisonalität im allgemein) in den Wochen vor der Blutprobe waren positiv korreliert mit Serum BDNF-Spiegel.

Diese Erkenntnisse erhöhen das verstehen von den Faktoren, die BDNF Expression regulieren. Außerdem sind die hier berichteten Ergebnisse von entscheidender Bedeutung in der Gestaltung und Auswertung von Studien zu BDNF.

TEIL II: der Neurotrophine Hypothese

Im **4. Kapitel** wir das Verständnis über die Zusammenhänge zwischen Serum BDNF-Spiegel und Depressionen erhöht. Daten zeigten das Serum BDNF-Spiegel niedriger sind in Antidepressivum freien Patienten in Bezug zu Kontrollen und Patienten die mit einem Antidepressivum behandelt wurden sein. Serum BDNF-Spiegels von Personen mit eine depressiven Erkrankung in vollständige Remission waren vergleichbar mit denen von gesunden Kontrolle Personen. Die Analysen zeigten weiter das BDNF-Spiegel nicht zusammenhängen mit der Schweregrad von Depression.

Dieses Paper zeigt, dass niedrige Serum BDNF-Spiegel ein Zustand Merkmal für Depression sind, die sich im Verlauf der natürlichen Remission und Behandlung mit einem Antidepressivum normalisiert. Wichtig ist, dass die Effekt-größen dieser Verbände klein sind und das die Normalisierung der Serum BDNF-Spiegel nicht unbedingt mit einer Entlastung von depressiven Symptomen zusammenhängt.

Kapitel 5 berichtet die Ergebnisse von mehreren Meta-Analysen auf Unterschiede im Serum BDNF-Spiegels in Antidepressivum freien depressiven Patienten im Vergleich zu gesunden Kontrolle Personen und mit Antidepressiva behandelten depressiven Personen. Das Paper zeigt niedrige Serum BDNF-Spiegels in Antidepressivum freien Patienten im Verhältnis zu gesunden Kontrollen und Antidepressiva behandelten Patienten. Wenn die Analysen für Publikations-bias kontrolliert werden dann scheinen die Effekt-größen über diese Verbände klein bis mittel-Groß am besten zu sein. Dieses Paper zeigt ferner, im Gegensatz zu früheren Überzeugung das Serum BDNF-Spiegels und die Schwere der Symptome der Depression nicht verwandt sind.

Dieses Papier ist bemerkenswert, nicht, dass es bestätigt dass Wechsel in Serum BDNF-Spiegels peripheren Manifestationen von Depression scheinen zu sein, aber dass es zeigt, dass der Beweis dafür wesentlich schwächer ist als ursprünglich angenommen wurde. Eine wichtige Implikation dieser Nachricht ist, dass Serum BDNF-Spiegels von wenig klinischen Einsatz sind.

In **Kapitel 6** forschen wir Serum BDNF-Spiegels in Patienten mit einer Angststörung und in gesunden Kontrollpersonen. Im allgemein fanden wir keine Unterschiede im Serum BDNF-Spiegels bei Patienten und Kontrollen. Eine Gender-Diagnose-Interaktion auf Serum BDNF-Spiegels wurde jedoch nachgewiesen. Diese Interaktion zeigt das weibliche Patienten mit einer Angststörung, niedrigere Serum BDNF-Spiegels hatten im vergleich zu weiblichen Kontrollen. Dies wurde nicht bei Männern beobachtet. Weitere Forschung zeigten das Serum BDNF-Spiegel nicht verwandt sind mit den klinischen Charakteristiken von Angst.

Angststörungen sind in vielen Aspekten ähnlich an Depression. Um diesen Grund war es zu erwarten, dass Serum BDNF-Spiegel niedrig waren bei Patienten mit einer solchen Krankheit. Außer etwas niedriger Serum BDNF-Spiegels bei weiblichen Patienten, könnte dieses Paper die Erwartung nicht bestätigen. Dieser Befund legt nahe, dass BDNF vielleicht eine Rolle spielt in der Pathophysiologie der Angst bei Frauen, oder, nicht unwahrscheinlich, einem weiblichen Artefakt von ängstlich sein beteiligt.

Kapitel 7 erforscht das vermuten das BDNF val^{66}met in Relation steht mit Serum BDNF-Spiegeln und ob dies abhängig ist von Kindheitstrauma oder andere Formen von Stress. Insgesamt war erfüllt das met Allel Träger niedrige Serum BDNF-Spiegel hatte, aber allein wenn sie zu Missbrauch in der Kindheit ausgesetzt werden. Außerdem, wenn nicht zu Missbrauch ausgesetzt, hatte met Allel Träger höhere Serum BDNF-Spiegels im Vergleich zu val/val Individuen (das ist eine val/met - Missbrauch Interaktionseffekt). Die Exposition zu Stressoren in der jüngsten Vergangenheit war mit einer kleinen Reduzierung der BDNF-Spiegels verbunden.

Trauma oder Stressbelastung ist nach der Neurotrophin Hypothese der Erklärung der niedrige BDNF-Spiegels in Personen mit einen depressive Erkrankung. Dieses Paper kann diese Erklärung nicht bestätigen. Außerdem berichtete das Paper eine val^{66}met - Kindheitstrauma Interaktionseffekt auf Serum BDNF-Spiegels. Die Ausmaß, in welche diese Interaktion wichtig ist (auf verschiedenen Ebenen der Funktion) benötigen zukünftige Forschung.

In **Kapitel 8** entwirren wir, ob Hippocampusvolumen/funktion und kognitive Leistung abhängig sind von Variationen in BDNF val^{66}met. Strukturelle und funktionelle MRI Daten wurden verwendet um diese aufzuklären. Unsere Ergebnisse zeigten etwas kleineren Hippocampus-volumen in Träger von einer met Allel relativ zu val/val Homozygoten. Für Hippocampus-kodierung Aktivität finden wir ein val^{66}met - Wort Valenz Wechselwirkung, so dass Träger eines met Allel erhöhte Aktivität zeigte in Reaktion auf emotional beladene Wörter.

Diese Ergebnisse, obwohl sie noch repliziert werden müssen, werfen die Frage auf, ob met Allel Träger eine abnormale Gehirnantwort auf emotionale beladen Stimuli zeigen. Neben den neurobiologischen Unterschiede beobachtet, konnte aber keine Effekte wurden gefunden auf Verhalten und Kognition.

Kapitel 9 enthält eine Meta-Analyse über die Assoziation zwischen BDNF val^{66}met und das Hippocampus-volumen. Die Ergebnisse zeigten, dass Träger eines met Allel niedriger Hippocampus-volumen hatten im vergeleich zu val/val Homozygoten. Zwischen-Studie Heterogenität in der Effekt-größen war aber sehr erheblich, und dies könnte nicht von demographischen, klinischen und methodischen Unterschiede zwischen den Studien erklärt werden. Wir fanden starke Hinweise auf Publikations-bias und Effekt-größen verringerten sich wesentlich über die Jahre. Diese alle zusammen zeigen dass niedrigere Hippocampus-volumen keine biologische Wirkung des met Allels sind, sondern ein methodisches Artefakt.

Der Erkenntnis der niedrige Hippocampus-volumen in Träger einer met Allel hat sich erfüllt wie eine Grundlage für die neurotrophinn Hypothese (gesamte Zitate nach das erste Papier auf dieses Vereins [Pezawas *et al.,* 2005] > 500). Die Meta-Analyse zeigt im Gegensatz, dass es nicht existiert und wahrscheinlich ein Artefakt ist von Studien mit unzureichende statistische power ist.

METHODEN

Bevor ich ihnen die Highlights der Diskussion und meine Schlussfolgerungen präsentiere, möchte ich die wichtigsten Stärken und Mängel der Methoden, die in dieser Arbeit verwendet wurden berichten, da diese bei der Interpretation der Ergebnisse sehr wichtig sind.

Eine deutliche hervorstechendes Stärke ist, dass die Studien in dieser Arbeit zureichende statistische power haben. Hiermit liefert diese These zuverlässige Schätzungen von wahre Effekt-Größen (Big Data Notable Fidelity). Eine weitere Stärke ist, dass eine hohe Validität erreicht wurde durch Anpassung für potenzielle Störfaktoren und durch Subgroup- und Moderation Analysen. Außerdem habe ich aktiv versucht

festzustellen, wie zuverlässig unsere Forschungsergebnisse waren durch die Durchführung von Meta-Analysen.

Ich bin mir trotzdem sehr bewusst von den Grenzen der Studien in dieser These. Zuerst sind unsere Schlussfolgerungen abhängig von periphere Maßnahmen um ein zentrales Prozess, die neuronale Plastizität, zu schätzen. Zweitens, diese These erarbeitet meist auf Querschnittsdaten und in keiner der Studien wurden die Teilnehmer nach dem Zufallsprinzip zugeordnet. Um diesen Grund sind die berichteten Ergebnisse nicht überzeugend genug um Kausalität zu beweisen. Zum Schluss sind die Ergebnisse in dieser These nicht (direkt) zu verallgemeinern auf alle Bevölkerungsgruppen, vor allem nicht auf den jungen, alten und schwer kranken.

DISKUSSION

Diese Arbeit hat zwei wichtige Elemente an der Literatur hinzufügen: (**I**) einen Überblick über die Determinanten des Serum BDNF-Spiegels und (**II**) es löst einige wichtige klinische Fragen in Bezug zu der Neurotrofe Hypothese. Zusammen haben diese Resultate erhebliche methodische und theoretische Implikationen, die im Folgenden zusammengefasst werden. Ich werde mit den Erkenntnissen über die Determinanten des Serum BDNF-Spiegels starten.

Teil I: Determinanten der Serum BDN-Spiegels

Das Verständnis der Determinanten eine Variable ist wichtig, um in der Forschung zu gültige Schlussfolgerungen zu kommen. Die ersten beiden Kapitel dieser Doktor Arbeit sind gewidmet zur Definition die Determinanten der Serum BDNF-Spiegels zu kommen.

Diese Arbeit beschreibt eine Liste von Variablen die systematisch mit Serum BDNF-Spiegels zugeordnet sind. Diese Liste enthält einige Variablen die, wenn nicht gut unter Kontrolle gehalten werden, einfach zum ungültige Schlussfolgerungen in die Prüfung der Vorhersagen. Zum beispiell, es ist eine Tatsache, dass die Prävalenz der Depression im Winter höher ist als im Sommer. Die starke Saisonalität im Serum BDNF-Spiegels (**Kapitel 3**), zusammen mit der höheren Prävalenz der Depression im Winter, deutet darauf hin, dass die Kontrolle für die Saison in denen die Probanden wurden getestet, wichtig sein kann, wenn man zum Beispiel der Unterschied im Serum BDNF-Spiegels zwischen depressiven Patienten und gesunden Kontrollen testet. Unsere Daten zeigen, dass dies in der Tat scheint so ist, da der Effektgröße überdiese Assoziation um 40 Prozent sinkt wenn eine für die Saison in denen die Probanden wurden getestet korrigiert.

Unter anderem aus diesem Grund, laden die Ergebnisse aus **Kapitel 2** und **3** ein zu eine Perspektive auf BDNF-Forschung, in welchen Determinanten integriert sind.

***Teil II:* der Neurotrofe Hypothese**

Serum BDNF-Spiegels - bestätigende Ergebnisse

Im Einklang mit der Neurotrofe Hypothese zeigen **Kapitel 4** und **5** das Serum BDNF-Spiegel niedrig sind in Antidepressivum freien depressiven Patienten in Bezug auf Kontrollen und depressiven Patienten die mit einem Antidepressivum behandelt wurden. Die Daten aus **Kapitel 4** und **5** zeigen weiter, dass die Serum BDNF-Spiegel von vollständig überwiesen Personen vergleichbar sind mit denen von gesunden Kontrollen. So niedrige Serum BDNF-Spiegels sind ein *Zustand Merkmal* von Depression: niedrig während der depressiven Zustand und normiert in voller Remission. Ebenfalls in Übereinstimmung mit was erwartet wurde, ist dass Serum BDNF-Spiegel normalisieren im Laufe der Behandlung mit Antidepressiva. Al diese Erkenntnisse sind robust, da sie von der größten einzige Studie (**Kapitel 4**) abgeleitet sind und durch Meta-Analysen (**Kapitel 5**) bestätigt wurden. Eine Erweiterung von der Neurotrofe Hypothese aus unsere Arbeit

ist das wir finden, dass weibliche Patienten mit einer Angststörung niedrigere BDNF-Spiegels haben im Verhältnis zu weiblichen Kontrollen (**Kapitel 6**). Dies heißt, dass BDNF in der Pathophysiologie von Angst bei Frauen beteiligt sein kann. Schließlich, und auch die Erwartungen bestätigend, zeigen wir dass die Exposition auf kürzliche belastende Ereignisse, *z. B.* eine Trennung (im allgemeinen einen ätiologischer Risikofaktor für die Entwicklung einer depressiven Erkrankung) im Zusammenhang sind mit einem (kleinen) Rückgang des Serum BDNF-Spiegels (**Kapitel 7**) .

Trotz dass diese Ergebnisse bestätigend sind, ist die Bedeutung von ihnen nicht immer klar (*z. B.* umgekehrte Kausalwirkung). Dabei zeigt ein Teil unserer Daten eine mangelnde Anpassung mit den Erwartungen aus der Neurotrofe Hypothese. Diese nicht bestätigende Ergebnisse werden diskutiert in der nachfolgenden Abschnitt.

Serum BDNF-Spiegel – nicht bestätigende Ergebnisse

Eine erste Feststellung, die nicht die Erwartungen von der Neurotrofe Hypothese folgt, ist das depressive Patienten, die in der frühen Remissionsphase ihrer Episode sind, und damit weitgehend beschwerdefrei waren, Serum BDNF-Spiegels hatte die niedriger sind im Vergleich zu den in derzeit depressiven Patienten (**Kapitel 4**). Diese Feststellung bezieht sich nicht gut auf die Erwartung der Neurotrofe Hypothese, dass niedrige neurotrofe Unterstützung ein Risiko für die Entwicklung einer Depression ist (*d. i.,* niedriges BDNF → Depression). In der Tat deutet diese Feststellung auf umgekehrten Kausalität, in dem niedrigere Serum BDNF Konzentrationen eine Folge sind von Depressionen (*d. i.,* Depression → niedrigen BDNF).

Weitere Ergebnisse in **Kapitel 4** und **5** , die sich nicht gut auf die Neurotrofe Hypothese beziehen, sind das BDNF-Spiegel nicht zu den klinischen Merkmale von eine Depressive Krankheit, wie der Schweregrad, verwandt sind und dass die Hochregulierung des Serum BDNF-Spiegels im Verlauf der antidepressiven Behandlung nur bei einer beschränkten Anzahl an Antidepressiva ein Effekt zeigt. Die Feststellung, dass BDNF-Spiegels und klinischen Merkmale von eine Depressiven Krankheit (bemerkenswerte die Schwere der Symptome) nicht Zusammenhängen, folgt nicht der Logik der linearen Dynamik der Neurotrofe Hypothese, aus dem es vorhergesagt werden kann das Patienten mit einem höheren Schweregrad der Symptome besonders niedrigen neurotrofe Unterstützung zeigen sollten. Bitte beachten Sie, dass in **Kapitel 5** dieser Arbeit, durch Meta-Analyse, die Abwesenheit von diesem Effekt robust bestätigt war. Die Feststellung von spezifischen Auswirkungen der Antidepressiva auf die Serum BDNF-Spiegels scheint im Widerspruch zu der Vorhersage der Neurotrofe Hypothese, dass ein erhöhter BDNF-Spiegel ein wichtiger Vermittler für ein Antidepressivum Reaktion ist. Nach dieser Vorhersage könnte man erwarten, dass Antidepressiva die in der Behandlung von Depressionen genau ebenso wirksam sind, ähnliche Auswirkungen auf die BDNF-Spiegel haben würden. Eine weitere und damit verbundene Feststellung die auch schwer mit der Neurotrofen Hypothese in Einklang zu bringen ist, ist dass die Gruppe der depressiven Personen die mit Antidepressiva behandelt war (für einen längeren Zeitraum und auf einer häufigen Basis) die höchsten BDNF-Spiegeln hatten neben dem höchsten Schweregrad der Depression (**Kapitel 4**). Dies heißt dass Erhöhungen in BDNF-Spiegels die offensichtlich im Verlauf der Behandlung auftreten, nicht parallel laufen mit die klinische Wirksamkeit von Antidepressiva.

Etwas das **Kapitel 5** addiert zu den oben erwähnten Verbänden ist, dass die Literatur weniger zuverlässig ist als wie man hoffen würde (*z. B.* Publikation-Bias). In der Tat, und im Gegensatz zu früheren Daten und Gewissen, sind die Effekt-Größen auf die Unterschiede zwischen den Gruppen nur klein und damit sind (Änderungen) in Serum BDNF-Spiegels wahrscheinlich von wenig klinische Verwendung (*z. B.* als Diagnostik Biomarker).

In **Kapitel 6** war die Erwartung, dass Serum- BDNF-Spiegel sind bei Menschen in einem ängstlichen Zustand. Die Daten ergaben jedoch wenig Grund zu dieser Annahme. Bei männlichen Patienten mit einer

Angststörung gab es sowieso keine Auffälligkeiten in Serum BDNF-Spiegeln. Bei weiblichen Patienten mit einer Angststörung fanden wir etwas niedrigere BDNF-Spiegeln im Vergleich zu gesunden Frauen. Daher werden die Daten in diesem Kapitel bezeugen, dass BDNF nicht in der Pathophysiologie der Angststörungen *per se* beteiligt ist. Und wie erklärt (*z. B.* in die Diskussion über das Verhältnis zwischen BDNF-Spiegels und Östrogen), kann die Veränderung bei weiblichen Patienten leicht eine bestimmte weibliche Artefakt bedeuten, ohne in die Angst Ätiologie ursächlich beteiligt zu sein.

Zu Letzt kann **Kapitel 7** die vorherrschende Axiom aus der Neurotrofe Hypothese bestätigten, dass Traumata die niedrige BDNF-Expression in der depressive Krankheit erklären.

Offenbar, Forschungsergebnisse die eine mangelnde Anpassung mit der Neurotrofe Hypothese hatten sind offensichtlich in unsere Studien. Ehrlich gesagt, konnten keine der Erwartungen die aus der Neurotrofe Hypothese abgeleitet werden können, voll und ganz bestätigt werden. Und wenn bestätigende Ergebnisse berichtet wurden, dann war oft ihrer Bedeutung nicht so klar (*z. B.* umgekehrte Kausalität). Vielleicht sind diese Ergebnisse selbst nicht eine ausreichende Disqualifikation von der Neurotrofe Hypothese doch zusammen schlagen sie vor, dass die ursprüngliche Idee nicht eindeutig eingehalten werden kann.

Die Neurotrofe Hypothese: BDNF val^{66}met

In den **Kapiteln 7** bis **9** erforschten wir Assoziationen zwischen einer Variante des Gens das für BDNF codiert, val^{66}met. Für die ist Funktionalität gezeigt (in vitro und in vivo) in Bezug auf neurotrofe Unterstützung. Wir haben ausdrücklich die Erwartung getestet, dass das met-Allel, das sogenannte Risiko-Allel an diesem Lokus, im Zusammenhang war mit Depressionen Phänotypen, von einer DSM-IV Depression Diagnose bis zur kognitive Funktion und Gehirnmorphologie. Wichtig ist das wir in unseren Studien Trauma und Stressbelastung sind enthalten neben deren mögliche Wechselwirkungen mit BDNF val^{66}met. Auf der Basis der vermuteten Funktionen in diesem Polymorphismus wurde es erwartet, dass die met Allel mit etablierten Korrelate der Depression verbunden werden sein soll, ins besonders im Angesicht der Trauma- und Stressbelastung.

BDNF val^{66}met - bestätigende Ergebnisse

Eine erste bestätigende Erkenntnis (**Kapitel 7**) war ein val^{66}met - Trauma Interaktions-Effekt auf die Serum BDNF-Spiegels. Dieses Effekt war so, dass BDNF met Allel Träger reduzierte Serum BDNF-Spiegel hatten, aber nur wenn sie ein Trauma erlebt hatten in der Kindheit. Darüber hinaus schienen Träger eines met Allels etwas niedrigere Hippocampus Volumen zu haben, bezogen auf val/val Homozygoten (**Kapitel 8**). Schließlich war die Hippocampus Aktivität in reaktion auf negativen Stimuli anders als Funktion der val^{66}met und Trauma-Exposition (**Kapitel 8**). Diese Ergebnisse sind im Einklang mit der Erwartung dass die met-Allel ein Risiko-Allel ist wenn an Depression verbündende Phänotypen als Ergebnis genommen wurde.

Trotz dieser Erkenntnisse gibt es in dieser Arbeit die notwendigen Befeindungen die nicht im Einklang scheinen zu sein mit den Vorhersagen der Neurotrofe Hypothese.

BDNF val^{66}met - nicht- bestätigende Ergebnisse

Befindungen die nicht im Einklang sein mit der Neurotrofe Hypothese sind unter anderem dass val^{66}met nicht (direkt) mit Psychopathologie, kognitive Leistung, Serum BDNF-Spiegel oder Encoding-Aktivität von der Hippocampus verbunden ist (**Kapitel 7** und **8**). Dabei scheint die Konstellationen der Ereignisse/Umstände, die einen Einfluss auf die Serum- BDNF-Spiegel und Hippocampus Encoding-Aktivität hatte (*d. h.* das met Allel und Exposition zu Missbrauch in der Kindheit) nicht mit den erwarteten Effekten auf der Verhalten verbunden waren (**Kapitel 7** und **8**). Schließlich, und vielleicht am meist schlüssig war die Studie in **Kapitel 9**, welche überzeugend demonstriert dass das val^{66}met nicht mit Hippocampus Volumen

verbunden ist. Das Studium im **Kapitel 9** ist auch im allgemeinen wichtig weil es die schädlichen Auswirkungen von Überschätzungen der Effekt-Größen (die diese und andere Forschungsfelder quälen) unterstreicht. Angesichts dieser nicht-bestätigende Befunde glaube ich, dass val^{66}met kein gültiges Modell ist um BDNF Funktion zu erforschen.

Abschluss

Das Vertrauen in eine Theorie nimmt zu, wenn sie von relevanten Daten bestätigt wird. Neben einigen konsistente Ergebnisse, zeigen unsere Daten (und auch die von vieler anderen) eine weitgehend fehlende universelle Bestätigung der Neurotrofe Hypothese. Und wo erwartete Ergebnisse gefunden werden, (*z. B.* ungewöhnlich niedrige Serum-BDNF-Konzentrationen im de Depressive Zustand), war die Bedeutung oft nicht so klar (*z. B.* umgekehrte Kausalität). Daraus schließe ich, während Nachteile unserer Studien anerkannt werden, dass die neuesten und zuverlässigsten Daten, die Neurotrofe Hypothese in seiner ursprünglichen Form nicht bestätigen - zurück an den Zeichentisch.

Zukunft der Arbeit - der Zeichentisch

Eine von den Zielen mit größter Bedeutung ist, um den Mechanismus des neurotrophen funktionieren im depressive Zustand kennen zu lernen (*d. h.* Konstruktvalidität) neben den funktionellen Konsequenzen auf die Gesundheit (*d. h.* Vorhersagevalidität). Daneben erfordert die zeitliche Dynamik wie die in der Neurotrofe Hypothese (*d. h.* geringe Unterstützung BDNF → Depression) beschrieben ist die nötige Forschung, weil nun umgekehrte Kausalität, in dem niedrigen BDNF Unterstützung nicht den Anfang markiert von einer depressiven Episode, sondern eine Folge davon ist, nicht ausgeschlossen werden kann (*d. h.* Konstruktvalidität). Weitere Herausforderungen die Notwendig sind um Fortschritte zu machen umfassen Zusammenarbeit und gemeinsame Nutzung von Daten um auf robuste Weise einige offene Fragen zu beantworten (*d. h.* statistische Validität), um einzelne BDNF Parameter zu messen, um die präklinische und klinische Forschung mehr zusammen zu bringen, und in parallel, die breite diagnostischen Kategorien des DSM in der Forschung von neurotrofe funktionieren zu verlassen (*d. h.* Konstruktvalidität). Sobald dies festgestellt werden kann, können Fortschritte gemacht werden, vielleicht am Ende begleitet von bisher nützliche Informationen für die klinische Bereichen.

RESUMEN ESPAÑOLES Y CONCLUSIONES

El papel de BDNF en la depresión

INTRODUCCIÓN

Apoyo neurotrófico es ubicuo en el cerebro donde es de importancia para el funcionamiento normal de la plasticidad neuronal, la memoria y la emoción. El neurotrófico Brain-Derived Neurotrophic Factor (BDNF) es el principal mediador de apoyo neurotrófico y se ha declarado que la comprensión de varias enfermedades psiquiátricas se puede aumentar cuando se entendiendo la regulación de esta hormona. De acuerdo con la *hipótesis de neurotrofina*, trastornos depresivos surgen de soporte neurotrófico aberrante por BDNF en áreas del cerebro que regulan la emoción. Con los años, esta hipótesis ha ganado apoya. Además, hay motivo para la creencia de que las medidas de periféricos (especialmente BDNF concentraciones en la sangre) y ciertas variantes genéticas (notablemente BDNF val^{66}met) pueden servir como ventanas para funcionamiento neurotrófico en el cerebro.

An medio de una gran expectación, sin embargo, hay dudas con respecto a las predicciones de la hipótesis neurotrofina. Fuentes de duda incluye una falta de conocimiento sobre los determinantes de las concentraciones séricas de BDNF y muchos preguntas clínicas sin respuesta. En esta tesis me dirigí exactamente a este fuentes de duda para crear un modelo (periférica) de funcionar BDNF más refinado en trastornos depresivos (y afines). Importante para mencionar es que en el trabajo actual las estimaciones del efecto sobre las asociaciones de interés se basan en estudios grandes y técnicas meta-analíticas.

Los datos empíricos que forman el corazón de esta tesis y una discusión sobre el se presentan en los capítulos anteriores. Un resumen detallado se presentará en la siguiente sección.

RESULTADOS

A continuación, se presentan los resultados de nuestros estudios empíricos (por capítulo) junto con la importancia que creo que pueden tener. El primer objetivo de esta tesis, delinear los determinantes de las concentraciones séricas de BDNF, se discribe en el **capítulo 2** y **3**. Los **capítulos 4** a **9** estaban destinados a las preguntas clínicas en relación con la hipótesis de neurotrofina.

PARTE I: determinantes

El **capítulo** 2 establece una descripción detallada de los determinantes de las concentraciones séricas de BDNF. Se nota en las personas que no fueron tratadas con antidepresivos y libre de una enfermedad psiquiátrica, que un estado de no-ayuno en el momento de la extracción de sangre, una disminución de sangre posterior en el día, y un mayor duración del almacenamiento de sangre se asociaron con concentraciones de suero BDNF atenuada. Esto fue en contraste con fumar y vivir en una zona urbana, que ambos se asociaron con un aumento de las concentraciones de BDNF. Por último, los sujetos de más edad tenían concentraciones de BDNF séricos más altos, pero esto aplican sobre todo a las mujeres (es decir, un efecto de la interacción entre edad y género).

La importancia de este trabajo es que da los determinantes de las concentraciones séricas de BDNF. Por lo tanto este estudio da una base mejorada para comprender las diferencias interindividuales en las concentraciones séricas de BDNF. Además, el conocimiento de los determinantes es esencial en la prevención de inferencias erróneas de los datos.

En el **capítulo 3** estudiamos arrastre estacional de las concentraciones séricas de BDNF. Los análisis por meses de muestreo mostraron una variación estacional pronunciada. Las concentraciones séricas de BDNF

aumentaron linealmente durante el período de primavera-verano (es decir, equinoccio de primavera) y disminuyeron linealmente durante el período otoño-invierno (es decir, Equinoccio de Otoño). Análisis exploratorios mostraron que la longitud natural del día y el número de horas de sol ambiente (principales factores para arrastrar estacionalidad) en las semanas previas a la extracción de sangre se correlacionó con las concentraciones séricas de BDNF.

Estos resultados se suman a la literatura, ya que ellos dan vías para comprender los factores que regulan la expresión de BDNF. Además, los resultados presentados en este documento son de vital importancia en el diseño y la evaluación de los estudios sobre el BDNF.

PARTE II: la hipótesis de la depresión neurotrofina

En el **cuarto capítulo** se avanza la comprensión de las asociaciones entre las concentraciones séricas de BDNF y depresión. Encontramos que concentraciones séricas de BDNF son bajos en los pacientes con depresión y libre de antidepresivos respecto a los controles y pacientes con depresión que fueron tratados con un antidepresivo. Las concentraciones séricas de BDNF de personas totalmente remitidos fueron comparables a los de sujetos sanos. Análisis también muestran que las concentraciones de BDNF no estaban relacionados con las características clínicas principales de la depresión, tales como su gravedad.

Este trabajo pone de manifiesto que las concentraciones de BDNF sérico son una característica del estado de depresión que se normaliza en el remisión natural y el tratamiento con antidepresivos. Además, este trabajo muestra que los tamaños del efecto sobre estas asociaciones son pequeñas y que la normalización de las concentraciones séricas de BDNF no se asocia necesariamente con un alivio de los síntomas depresivos.

Capítulo 5 presenta los resultados de varios meta-análisis sobre las diferencias en concentraciones de BDNF en pacientes con depresión no tratados de antidepresivos, sujetos control sanos y personas con depresión tratados con antidepresivos. El estudio muestra concentraciones de BDNF sérico bajo en pacientes no tratados de antidepresivos en relación con controles sanos y pacientes tratados con antidepresivos. Cuando *publicación-bias* se contabilizó, los tamaños del efecto se convirtieron en pequeña a mediana. Este papel muestra además, en contraste con la creencia previa, que concentraciones de BDNF y la gravedad de la depresión no están relacionados.

Este trabajo es digno de mención, no en que confirma que alteraciones en las concentraciones de BDNF parecen ser manifestaciones periféricas de depresión, pero que muestra que la evidencia de esto es más delgada como se pensaba inicialmente. Una implicación importante de este mensaje es que las concentraciones del BDNF probablemente son de poca utilidad clínica.

En el **capítulo 6** se evaluaron concentraciones de BDNF en pacientes con un trastorno de ansiedad y en controles sanos. Generalmente no encontramos diferencias en las concentraciones séricas de BDNF entre pacientes y controles. Una interacción género-diagnóstico sin embargo se detectó que indica que los pacientes de sexo femenino con un trastorno de ansiedad tenían menores concentraciones séricas de BDNF in relación con los controles de sexo femenino. Esto no se observó en los hombres. Un hallazgo adicional fue que los concentraciones de BDNF no estaban relacionados con las características clínicas de la ansiedad.

Los trastornos de ansiedad imitan la depresión en gran medida, por lo que se espera que concentraciones séricas de BDNF serían bajos en pacientes con esa enfermedad. Este documento no confirma eso expectativa, excepto que las concentraciones del BDNF estaban algo inferiores en pacientes de sexo femenino. Este hallazgo puede sugerir que el BDNF está implicado en la fisiopatología de la ansiedad en las mujeres o, no improbable, un artefacto específico femenino de ser ansioso.

El **séptimo capítulo** se dirigió a la presunta efecto del BDNF val^{66}met en BDNF concentraciones, y si, o está efecto es condicionado a la exposición al trauma infantil o otras formas de estrés. En general, portadores de una met alelo tenian reducido BDNF concentraciones cuando expuestos al abuso en la niñez. Ademas, cuando no expuesto a abusos en la infancia, portadores de una met alelo tenian BDNF concentraciones superiores a val/val individuos (es decir, un val/met- abuso en la niñez efecto de la interacción). La exposición de estrés recientes se asoció con una pequeña disminución en las concentraciones de BDNF.

Trauma o estrés exposición son acordeó a la hipótesis de neurotrofina, el axioma que explique BDNF concentraciones reducidos en personas con depresión. Este documento no confirma esta idea. Aquí se hace reportar un val/met - abuso efecto de la interacción en las concentraciones séricas de BDNF. El grado en que esta interacción puede ser importante (en varios niveles de funcionamiento) será explorado en la investigación futura.

Capítulo 8 desentrañar si el volumen y el funcionamiento del hipocampo y el rendimiento cognitivo están relacionados con variación en BDNF val^{66}met. MRI datos estructurales y funcionales mostraron volúmenes del hipocampo ligeramente más pequeñas en los portadores de un met alelo en relación con los homocigotos val/val. Para la actividad del hipocampo codifica encontramos una interacción de valencia de estimulos y val^{66}met tales que los portadores de un met alelo se mostraron mayores niveles de actividad en respuesta a las palabras emocionales.

En este trabajo se promueve la comprensión de la asociación de BDNF val^{66}met con el volumen/funcionamiento del hipocampo y el rendimiento cognitivo. Encontramos un pequeño efecto de val^{66}met en el volumen del hipocampo. Tambien encontramos que el abuso de la niñez explica diferencias individuales en la actividad de la codificación del hipocampo. Este último efecto se manifiesta de forma diferente en función de val^{66}met. Estos resultados, aunque en necesidad de replicación, plantear la cuestión de si los portadores de un met alelo muestran una respuesta abnormal del cerebro en los estímulos emocionales cargados. Este mensaje viene con la noción de que no se observaron efectos en el comportamiento junto con las diferencias neurobiológicos.

Capítulo 9 contiene una revisión y meta-análisis de la asociación entre el BDNF val^{66}met y el volumen del hipocampo. Los resultados mostraron que los portadores de un met alelo tenían volúmenes del hipocampo algo más bajos en relación con los homocigotos val/val. Heterogeneidad entre los estudios en las estimaciones del tamaño del efecto fue considerable y esto no se puede explicar por las diferencias demográficas, clínicas y metodológicas entre los estudios. Hemos encontrado una fuerte evidencia de publicación-bias y los tamaños del efecto se redujeron considerablemente en los últimos años. Todo esto llevó a la conclusión de que los volúmenes de hipocampo inferiores no son un verdadero efecto biológico del met alelo, sino más bien un artefacto metodológico.

La idea de que los volúmenes del hipocampo son bajas en portadores de un met alelo se ha convertido en un pilar para la hipótesis neurotrofina (total de citas para el primer artículo sobre esta tema [Pezawas *et al.,* 2005] es más de 500). El meta-análisis sobre este tema se muestra que esta asociación es inexistente y probablemente un artefacto debido a la utilización de estudios de poca potencia.

MÉTODOS

Antes de presentar los aspectos más destacados de la discusión y mis conclusiones, presentar*é* en breve las principales fortalezas y limitaciones de los métodos que se he utilizado en esta tesis ya que deben reflejarse en estos cuando interpretar los resultados se presente en este Thesis.

Una fortaleza más destacada es que, básicamente todos los estudios en esta tesis son basados en estudios de mucha potencia estadistica. Por eso presentamos estimaciones del tamaño del efecto confiables (Big Data Notable Fidelity). Otro punto fuerte es un nivel de validez alto por que los resultas se logró estaba ajuste para posibles factores de confusión y analysis de subgrupos y moderación. Además, yo busqué activamente para determinar el grado de fiabilidad de nuestros resultados a cabo de meta-análisis.

También estoy muy consciente de las limitaciones de los estudios en mi tesis. En primer lugar, nuestras conclusiones están supeditadas a las medidas de periféricos para medir un proceso central: de la plasticidad neuronal. En segundo lugar, esta tesis elaborada principalmente en datos *cross-sectional* y en ninguno de los estudios los sujetos fueron asignados *ad random* a las condiciones en que se encontraban. Por lo tanto los resultados reportados no son suficientemente convincentes para demostrar causalidad. Por último, los resultados en este documento no se generalizan (directamente) a todas las poblaciones, en particular, no a los jóvenes, los viejos y los enfermos graves.

DISCUSIÓN

En esta tesis logramos dos cosas importantes: (**I**) presentamos los determinantes de las concentraciones séricas de BDNF y (**II**) resolvemos algunas preguntas clínicas con respecto a la hipótesis de la neurotrofina. En conjunto, estos logros tienen implicaciones metodológicas y teóricas importantes que se resumen a continuación. Comenzo con las conclusiones sobre los determinantes de las concentraciones de BDNF.

Parte I: determinantes de los niveles séricos de BDNF

Comprensión sobre determinantes de una variable es vital para venir a conclusiones válidas. Los dos primeros capítulos de esta tesis se describen los resultados de nuestros esfuerzos para comprender los determinantes de los niveles séricos de BDNF.

En esta tesis se descubre una serie de variables que se asocian sistemáticamente con los niveles séricos de BDNF, incluye algunas que cuando no se controlar adecuada, se puede venir fácilmente a conclusiones no válidas. Un ejemplo que ilustra esto bien es el efecto estacional de los niveles séricos de BDNF (**Capítulo 3**). Es un hecho que la prevalencia de la depresión es mayor en invierno en comparación con el verano. La estacionalidad en los niveles séricos de BDNF que nosotros encontramos, junto con la mayor prevalencia de depresión en el invierno, sugiere que uno debe controlarse para la temporada cuando se estudia, por ejemplo, la diferencia en los niveles séricos de BDNF entre los pacientes deprimidos y controles sanos. Nuestros datos muestran que esto parece ser cierto, ya que el tamaño del efecto disminuye por no menos de 40 por ciento cuando los análisis están controlada para la temporada en la que se hicieron las mediciones.

Así, nuestro grupo ha aumentado la comprensión de los factores determinantes de los niveles séricos de BDNF. Y claro, todos los hallazgos de **Capítulo 2** y **3** invitar a una perspectiva en que estos determinantes están integrados.

Parte II: determinantes de los niveles séricos de BDNF

Las concentraciones séricas de BDNF - observaciones confirmatorias

En la línea con lo que se esperaba, el **capítulo 4** y **5** muestran que las concentraciones séricas de BDNF son bajos en los pacientes con depresión no tratados de antidepresivos al respecto a los controles y los pacientes con depresión que fueron tratados con un antidepresivo. Los datos de **capítulo 4** y **5** además indican que las concentraciones séricas de BDNF de personas totalmente remitidos son comparables a los de controles sanos. Así, las concentraciones de BDNF sérico bajo son una característica de *estado* de depresión: evidente durante el estado de depresión y normalizó en remisión completa. También en la línea

de lo que se esperaba es que las concentraciones séricas de BDNF se normalizan en el curso del tratamiento antidepresivo. Todos estos resultados son robustos, ya que se derivan de la estudio más grande (**capítulo 4**) y son confirmados por meta-análisis (**capítulo 5**). Además, encontraron una extensión de la hipótesis de neurotrofina. Es que las pacientes mujeres con un trastorno de ansiedad tienen menores concentraciones de BDNF en relación con controles sanos (**capítulo 6**). Por eso, BDNF podría estar implicado en la fisiopatología de la ansiedad en las mujeres. Por último , y también siguiendo la expectativa es que la exposición a de estrés recientes, como ser despedido (un factor de riesgo general para una enfermedad depresiva), se asoció con una (pequeña) disminución de las concentraciones séricas de BDNF (**capítulo 7**) .

Aunque estos resultados eran confirmatoria con el hipótesis de neurotrofina, que exactamente significa no siempre eran tan claro (por ejemplo, los resultados pueden significar la causalidad inversa, para ser discutido). Además, una parte de los datos sobre las concentraciones de BDNF no se confirmó a las expectativas de la hipótesis de neurotrofinas. Los hallazgos no confirmatorias están el tema de la parte que sigue.

Las concentraciones séricas de BDNF – observaciones non confirmatorias

Una primera constatación que no está en línea con las expectativas que se encontraban en este tesis es que los pacientes con depresión en la fase de remisión temprana de su episodio depresivo, y por lo tanto en gran medida libres de síntomas, tenían concentraciones séricas de BDNF más bajos en comparación con pacientes con una depresión actualmente (**capítulo 4**). Así, este hallazgo no se refiere a la dinámica temporal especificados en la hipótesis de neurotrofina que el apoyo neurotrófico baja debe poner en peligro a convertirse en depresión (es decir, bajo el BDNF→ depresión). De hecho, se sugiere la causalidad invertida, donde las concentraciones de BDNF inferiores son una consecuencia de la depresión (es decir, depresión → bajo BDNF).

Otros hallazgos reportados en el **capítulo 4** y **5** que relatan mal con la hipótesis neurotrofina es que BDNF concentraciones no se relacionan con las características clínicas de la depresión, tales como su gravedad y que la regulación de BDNF en el curso del tratamiento con antidepresivos se limita a algunas clases de antidepresivos. El hallazgo sobre la falta de una relación entre las concentraciones de BDNF y las características clínicas (notable el gravedad) no confirma la lógica lineal de la hipótesis de neurotrofina, de la que se puede predecir que los pacientes con mayor gravedad de los síntomas muestran apoyo neurotrófico particularmente baja. Por favor, tenga en cuenta que el **capítulo 5**, un meta-análisis, confirma la ausencia de la asociación entre las concentraciones séricas de BDNF y gravedad de depresión (una asociación en la que la literatura tenía mucha fe). El hallazgo de los efectos específicos de antidepresivos en las concentraciones del BDNF contraste con la predicción de la hipótesis de la neurotrofina de que el aumento de BDNF es un mediador clave de la respuesta a una antidepresiva. De acuerdo con esta predicción se podría esperar que los antidepresivos, que son igualmente eficaces en el tratamiento de la depresión, tendrían efectos similares en las concentraciones de BDNF, que (de acuerdo con nuestros datos, sino también la de los demás) obviamente no tienen. Otro hallazgo que es difícil de conciliar con la hipótesis de neurotrofina es que el grupo de personas con depresión que utilizaron antidepresivos (por un período prolongado y sobre una base frecuente) tuvieron los más altos concentraciones de BDNF junto a una gravedad de depresión más alta (**capítulo 4**). El significa de este hallazgo debe ser que los aumentos en las concentraciones de BDNF periféricas que ocurren en el curso del tratamiento no van paralela con la efectividad clínica de los antidepresivos.

Qué **capítulo 5** añade con respecto a las asociaciones mencionadas es que la literatura es menos fiable como se podía esperar (por ejemplo, el bias de publicación). De hecho, y en contraste con los datos

anteriores y de creencias, Los pequeños estimaciones del tamaño indican además que (cambios en) las concentraciones séricas de BDNF son de poco uso clínico como, por ejemplo, marcador diagnóstico o tratamiento.

En el **capítulo 6** esperaba que las concentraciones séricas de BDNF serían abnormalmente bajo en el estado de ansiedad. Los datos, sin embargo, no apoyó esta creencia. En los pacientes masculinos con un trastorno de ansiedad no había observada anormalidades en la concentración sérica de BDNF. Sin embargo, nos dimos cuenta de que en las mujeres con un trastorno de ansiedad encontramos concentraciones séricas de BDNF ligeramente bajo en comparación con las mujeres sanas. Por lo tanto, los datos de este capítulo dan fe en el creencia de que el BDNF no está implicado en la fisiopatología de los trastornos de ansiedad. Y, como he explicado (véase la discusión sobre la relación entre las concentraciones séricas de BDNF y estrógenos), las alteraciones en las pacientes femenina pueden ser fácilmente un artefacto específico de estar ansioso sin estar causalmente implicado en la etiología de la ansiedad.

Finalmente, el **capítulo 7** atestigua el axioma predominante de que se ha adelantado en la explicación de las alternancias en la expresión de BDNF relacionados con la depresión: la exposición al trauma.

Claramente, resultados que muestran una falta de ajuste con las expectativas de la hipótesis de neurotrofina son omnipresentes en los estudios que componen esta tesis. Francamente, ninguna de las expectativas que se pueden derivar de este hipótesis se pudo validar plenamente. Y si nos reportamos hallazgos confirmatorias, su respectivo significado no siempre era tan clara (por ejemplo la causalidad inversa). Tal vez estos hallazgos no confirmatorias por no son suficiente para rechazar la hipótesis neurotrofina todavía sí misma, pero juntos significan en mi opinión que la idea inicial de la hipótesis de neurotrofina ya no debe ser acreditado.

La hipótesis de neurotrofina: BDNF val^{66}met

Los **capítulos 7** a **9** exploraron las asociaciones entre una variante en el gen que codifica para el BDNF, val^{66}met, para los que funcionalidad se ha demostrado (*in vitro* e *in vivo*) en términos de apoyo neurotrófico. Pruebamos explícitamente la expectativa de que el met alelo, el llamado alelo de riesgo en este lugar, se relaciona con la depresión y fenotipos relatos. Una nota importante es que la exposición al trauma y el estrés se han tenido en cuenta en estos estudios con el fin de modelar las diferencias interindividuales debido a estos factores y su posible interacción con BDNF val^{66}met. Sobre la base de la supuesta funcionalidad de este polimorfismo se esperaba que el met alelo estaría asociados con correlaciones establecidas de la depresión, particularmente cuando un persona también estaba expuesto al trauma y/o otras formas del estrés

BDNF val^{66}met - observaciones confirmatorias

Un primer hallazgo confirmatorio (**capítulo 7**) fue un efecto de interacción entre val^{66}met y trauma en las concentraciones séricas de BDNF. Este efecto fue tal que portadores de un met alelo habían concentraciones de BDNF reducido, pero sólo cuando se expone al eventos traumaticos. En contraste con las expectativas esto no tiene ningún efecto sobre el comportamiento. Por eso el grado en que este interaction es importante no se ha dilucidado. Además, portadores de un met alelo parecía tener volúmenes del hipocampo algo más bajos en relación con los homocigotos val/val (**capítulo 8**). Por último, la actividad del hipocampo durante la recuperación de estimulas negativas fue diferente en función de val^{66}met y la exposición al trauma (**capítulo 8**). Estos observaciones pueden estar en línea con la expectativa de que el alelo met es un alelo de riesgo cuando se toman los fenotipos relacionados con la depresión como medido de resultado.

Sin embargo, también en relación con val^{66}met esta tesis reporta resultados que divergen de las predicciones de la hipótesis de las neurotrofina. Estas inconsistencias se detallan a continuación .

BDNF val^{66}met - observaciones no confirmatorias

Los resultados que no estaban en consonancia con la creencia incluidos que val^{66}met no era (directamente) asociado con psicopatología, funcionamiento cognitivo, las concentraciones séricas de BDNF, o los patrones de activación del hipocampo (**capítulo 7** y **8**). Además, da cuenta que las constelaciones de eventos/circunstancias que tuvieron un efecto sobre las concentraciones séricas de BDNF y la actividad de la codificación del hipocampo (el met alelo y la exposición al trauma) no se asociaron con resultados esperados a nivel conductual (**capítulo 7** y **8**). Finalmente, y tal vez más concluyente, **capítulo 9** muestra que val^{66}met no está asociado con el volumen del hipocampo. Este estudio es importante en general por que pone los efectos nocivos de los estudios de poca potencia y sobre-estimaciones de los tamaños del efecto. Por eso el papel puede ser relevante más allá del val^{66}met literatura. Dados estos hallazgos no confirmatorias, creo que val^{66}met no es un modelo válido para estudiar el funcionamiento del BDNF en humanas.

Conclusión

La confianza en una teoría aumenta cuando se confirma con datos pertinentes. Junto con algunos hallazgos consistentes, nuestros datos (y también la de los demás) muestran en gran falta de confirmación universal respecto a la hipótesis de la neurotrofina. Y dónde se establecieron asociaciones esperados (por ejemplo, concentraciones de BDNF abnormalmente bajo en el estado de depresión), el significado no era tan clara a menudo (por ejemplo, la causalidad inversa). Por lo tanto, llego a la conclusión, teniendo en cuenta las limitaciones y reconociendo que los resultados están supeditados a parámetros imperfecta, que la evidencia reciente y más confiable en humanos no corrobora la hipótesis neurotrofina y por eso ya no debe ser acreditado en su forma original - volver a la mesa de dibujo.

El trabajo futuro - la mesa de dibujo

En la mesa de dibujo se me ocurrió algunos objetivos importantes. El uno de estos con mayor importancia es profundizar la comprensión de cómo el funcionamiento neurotrófico puede ser modificada en el estado de depresión (es decir, la validez de constructo) y las consecuencias funcionales de este en la salud (es decir, la validez predictiva). Junto a esto, la dinámica temporal como se especifica en la hipótesis de neurotrofina (es decir, bajo apoyo BDNF → inicio de la depresión) deben ser atrapados, porque la causalidad invertido (es decir, depresión → apoyo bajo BDNF) en la que el apoyo bajo BDNF no marca el comienzo de un episodio depresivo, sino más bien una consecuencia ahora no se pueden excluir (es decir, la validez de constructo). Otros desafíos en el camino hacia progreso incluyen la colaboración en redes de intercambio de datos para resolver, con rigor, algunas cuestiones pendientes (es decir, de validez estadística), para medir más allá de los parámetros individuales de BDNF, para llevar la investigación preclínica y clínica más juntos, y, en paralelamente, para dejar atrás las categorías diagnósticas del DSM en el estudio de neurotrófico funcionamiento (es decir, validez de constructo). Cuando esto se puede establecer, se puede avanzar, tal vez en el final acompañado de la información útil para áreas clínicas.

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Dan de andere specialisten die op mijn pad gekomen zijn en met wie ik heb mogen werken: Anna Kaimatzoglou, Brisa Fernandes, Judith Haffmans, Marie-Jose van Tol, Maryna Polyakova, Mirjam Polak, Andre Aleman, Boudewijn Bus, Dick Veltman, Gunter Kenis, Jos Prickaerts, Marinus van IJzendoorn, Nic van der Wee en Richard Oude Voshaar -- dank jullie wel voor al de kennis die jullie met mij hebben willen delen en voor jullie ondersteuning.

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Zij past in het vorige rijtje – maar krijgt er een apart: ¡Anouk! – bedankt voor de voor- en de achterkant van dit boekwerk maar vooral voor ons samenzijn en het geluk dat je mij geeft.

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Curriculum Vitae, Nederlandse versie

Na zijn middelbare school (Maranatha Boskoop) heeft Marc Molendijk (Gouda, 27 Juni 1974) zijn dienstplicht vervult bij de Koninklijke Marine. Hier beviel het hem zozeer dat hij er nog eens 8 jaar bleef. Ten tijde van zijn terugkeer in het burger bestaan, 28 jaar oud, schreef hij zich in bij de Universiteit van Leiden alwaar hij in 2008, na het behalen van twee Master titels, onder leiding van Bernet Elzinga als promovendus aan de slag mocht. Zijn samenwerking met Bernet *c.s.* heeft geleid tot het onderhavige boekwerk. Naast zijn onderzoekswerk vervulde Marc in de afgelopen jaren, in deeltijd, een functie als docent aan de Universiteit Leiden en werkte hij enige tijd in de ambulante behandeling van mensen met een depressie in Den Haag. Recent heeft Marc in Leiden een positie als Universitair docent/post-doc onderzoeker weten te bemachtigen. Ook is hij in dienst als onderzoeker bij PsyQ in Den Haag. Zijn onderzoek naar BDNF, maar ook dat naar andere onderwerpen, heeft hem in zijn greep en hij wil graag voort stomen op dit uitdagende pad.

Curriculum Vitae, English version

After completing secondary school (Maranatha Boskoop), Marc Molendijk (Gouda, 27 June 1974) performed his military duty in the Dutch Royal Navy where he stayed for a total of nine years. Upon his return to civil society, at age 28, he entered Leiden University where he, in 2008, graduated for two Master degrees. After this he applied for a PhD position under the supervision of Bernet Elzinga *c.s*. He got the job, embraced it with eagerness and eventually wrote this thesis that you maybe are about to read. While working on his thesis, Marc held a part-time educational position at Leiden University and he worked at a depression outpatient's unit in The Hague. Recently he successfully applied for a position as assistant professor/post-doc researcher in Leiden and he holds a position as part-time researcher at PsyQ The Hague. His research on BDNF, but also that on other topics, is exciting enough for him to forge ahead on this path.

List of collaborators (in alphabetical order)

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MSc. Anna Kaimatzoglou	Edinburgh	Scotland
Prof. dr. Bernet M Elzinga	Leiden	The Netherlands
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List of publications (in chronological order)

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APPENDIX I

Chapter 1 General introduction

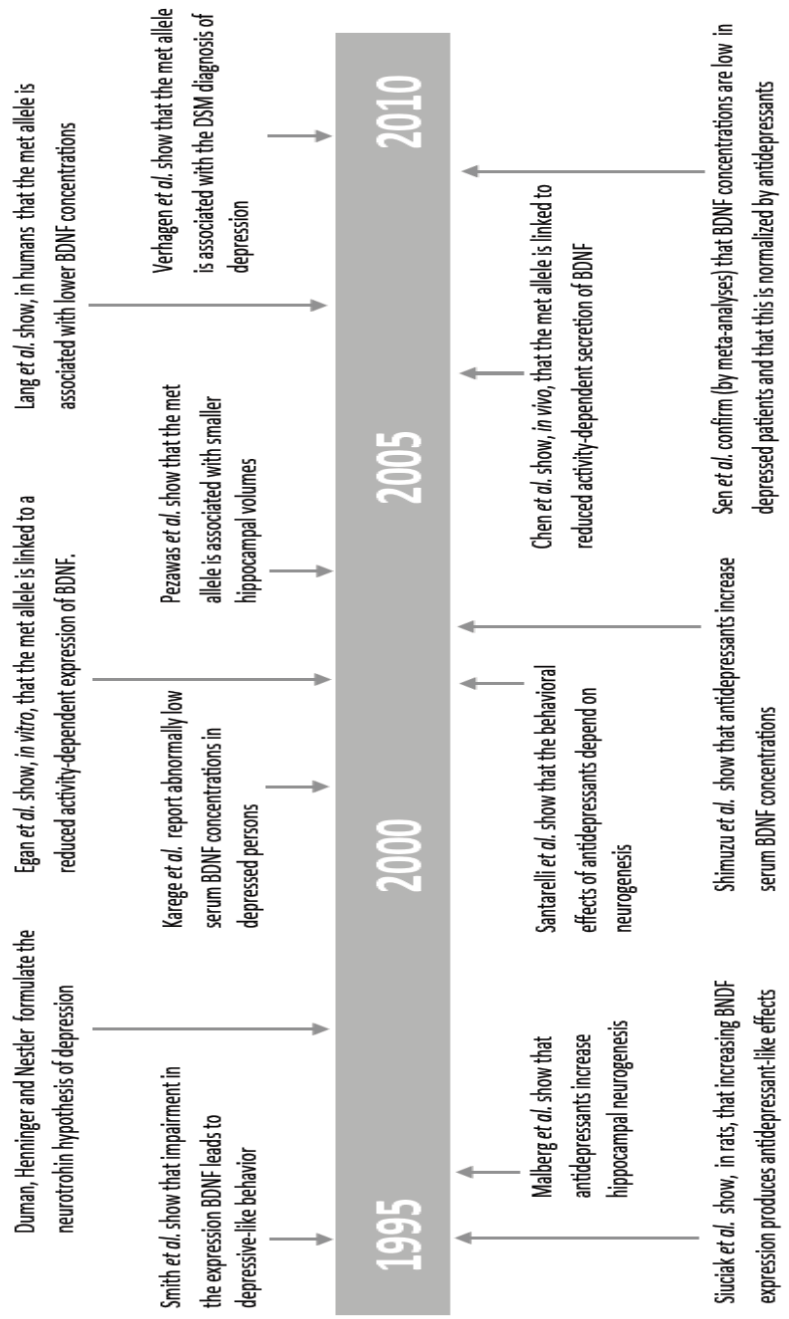

Figure S1. Overview of the breakthroughs in the research into the neurotrofin hypothesis

APPENDIX II

Chapter 3 BDNF concentrations show strong seasonal variation and are correlated with the amount of ambient sunlight

Table S1. *P*-values for pair-wise comparisons on covariate adjusted serum BDNF concentrations by month of sampling

	Jan *n* = 249	Feb *n* = 238	Mar *n* = 239	Apr *n* = 228	May *n* = 229	Jun *n* = 231	Jul *n* = 203	Aug *n* = 211	Sep *n* = 280	Oct *n* = 254	Nov *n* = 292	Dec *n* = 197
Jan	1	.19	.01 ↑	.74	.26	.001 ↓	.003 ↓	.001*↓	<.001*↓	.004 ↓	<.001*↓	.003 ↓
Feb	.19	1	.21	.35	.02↓	<.001*↓	<.001*↓	<.001*↓	<.001*↓	<.001*↓	<.001*↓	<.001*↓
Mar	.01 ↓	.21	1	.03 ↓	<.001*↓	<.001*↓	<.001*↓	<.001*↓	<.001*↓	<.001*↓	<.001*↓	<.001*↓
Apr	.74	.35	.03 ↑	1	.15	<.001*↓	.001 ↓	<.001*↓	<.001*↓	.001 ↓	<.001*↓	.002 ↓
May	.26	.02 ↑	<.001*↑	.15	1	.04 ↓	.06	.02 ↓	.001 ↓	.09	.006 ↓	.07
Jun	.001 ↑	<.001*↑	<.001*↑	<.001*↑	.04 ↑	1	.92	.78	.27	.69	.59	.87
Jul	.003 ↑	<.001*↑	<.001*↑	.001 ↑	.06	.92	1	.72	.25	.78	.53	.94
Aug	.001*↑	<.001*↑	<.001*↑	<.001*↑	.02 ↑	.78	.71	1	.44	.50	.81	.66
Sep	<.001*↑	<.001*↑	<.001*↑	<.001*↑	.001 ↑	.27	.25	.44	1	.12	.56	.22
Oct	.004 ↑	<.001*↑	<.001*↑	.001 ↑	.09	.69	.78	.50	.12	1	.33	.84
Nov	<.001*↑	<.001*↑	<.001*↑	<.001*↑	.006 ↑	.59	.53	.82	.56	.33	1	.48
Dec	.003 ↑	<.001*↑	<.001*↑	.002 ↑	.07	.87	.94	.66	.22	.84	.48	1

* Statistically significant after Bonferroni correction was applied (66 comparisons, critical *P* value = .00076)
↑ Higher serum BDNF levels in the month indicated in the row relative to the month indicated in the corresponding column
↓ Lower serum BDNF levels in the month as indicated in the row relative to the month as indicated in the corresponding column

Table S2. Zero-order and partial Pearson's correlation coefficients with corresponding *P*-values on the associations between the number weekly sunlight hours and serum BDNF concentrations

	Zero-order correlation	*P*-value	Partial correlation [1]	*P*-value
Number of sunlight hours in the:				
Week of blood draw	0.03	.08	0.04	.03
Week prior to blood draw	0.03	.07	0.04	.04
Two weeks prior to blood draw	0.02	.11	0.04	.03
Three weeks prior to blood draw	0.04	.01	0.06	.001
Four weeks prior to blood draw	0.07	< .0001	0.09	< .0001
Five weeks prior to blood draw	0.12	< .0001	0.13	< .0001
Six weeks prior to blood draw	0.14	< .0001	0.15	< .0001
Seven weeks prior to blood draw	0.15	< .0001	0.16	< .0001
Eight weeks prior to blood draw	0.16	< .0001	0.18	< .0001
Nine weeks prior to blood draw	0.15	< .0001	0.16	< .0001
Ten weeks prior to blood draw	0.12	< .0001	0.13	< .0001

[1] See the paper for covariates

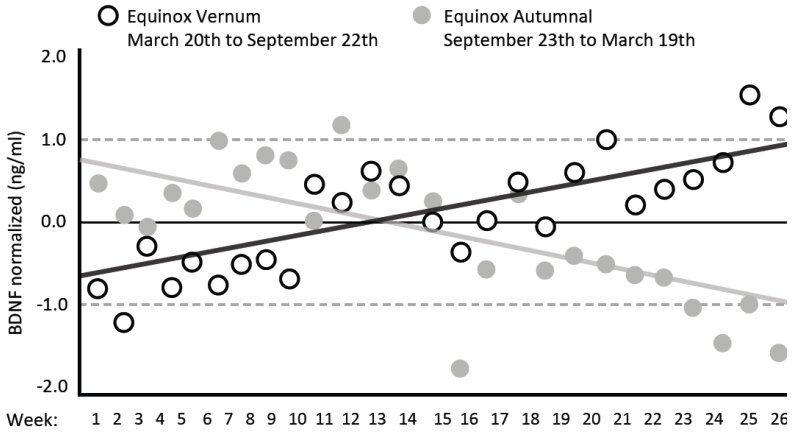

Figure S1. Mean normalized serum BDNF concentrations plotted as a function of each consecutive week of measurement in each equinox

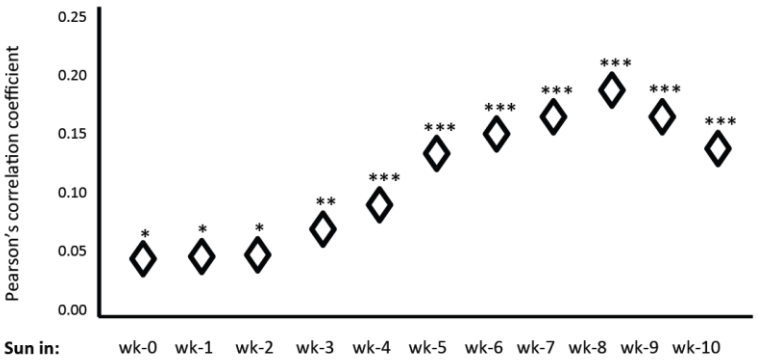

Figure S2. Partial Pearson's correlation coefficients (for covariates see the paper) on the relation between mean serum BDNF concentrations and the hours of sunlight in the week of blood draw (wk-0) and the 10 weeks prior to blood draw (wk-1 to wk-10). * $P < .05$, ** $P < .001$, *** $P < .0001$

APPENDIX III

Chapter 5 Serum BDNF concentrations as peripheral manifestations of depression: *evidence from a systematic review and meta-analyses on 179 associations (N = 9,484)*

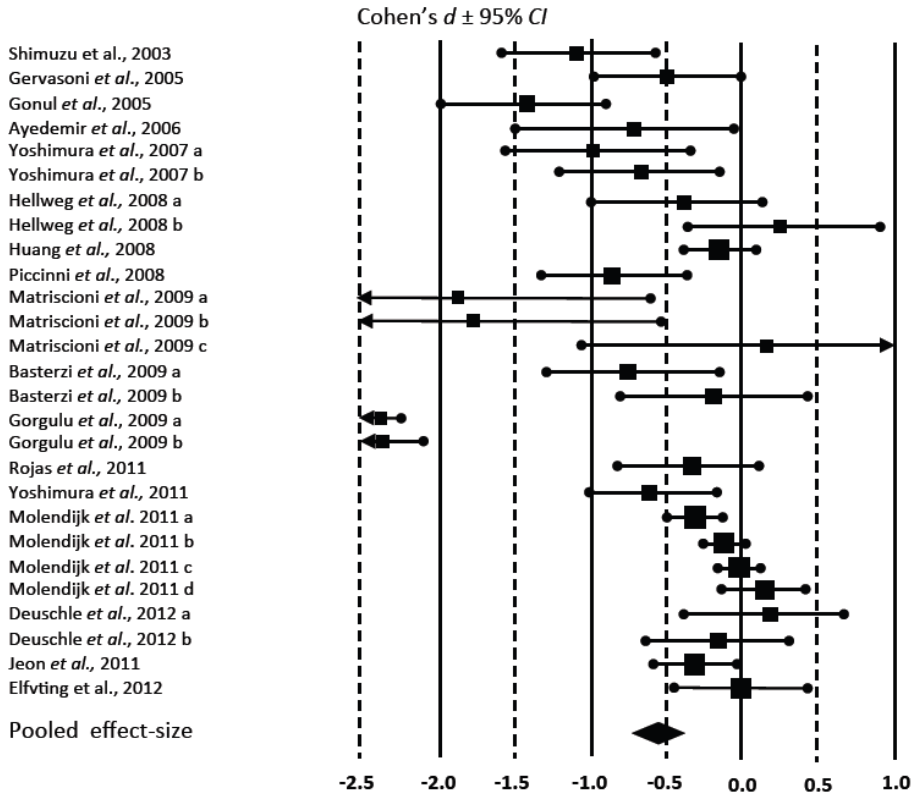

Figure S1. Forest plot for random effect meta-analysis on differences in serum BDNF concentrations between antidepressant-free and antidepressant treated depressed patients. The sizes of the squares are proportional to sample size.

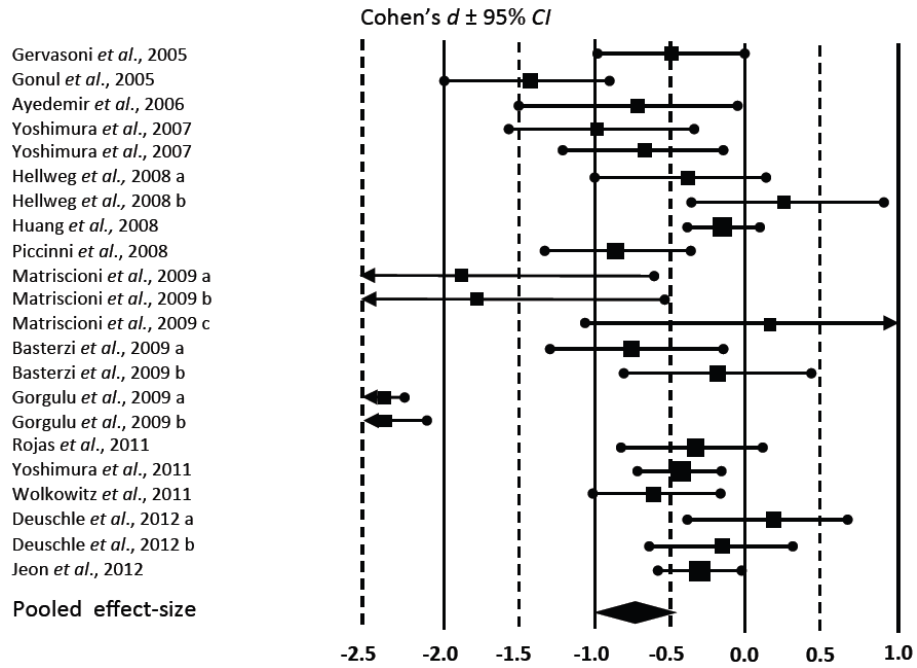

Figure S2. Forest plot for random effect meta-analysis on differences in serum BDNF concentrations between antidepressant-free and treated depressed patients (within-subjects data only, that is treatment studies applying a pre- and post-treatment design). The sizes of the squares are proportional to sample size.

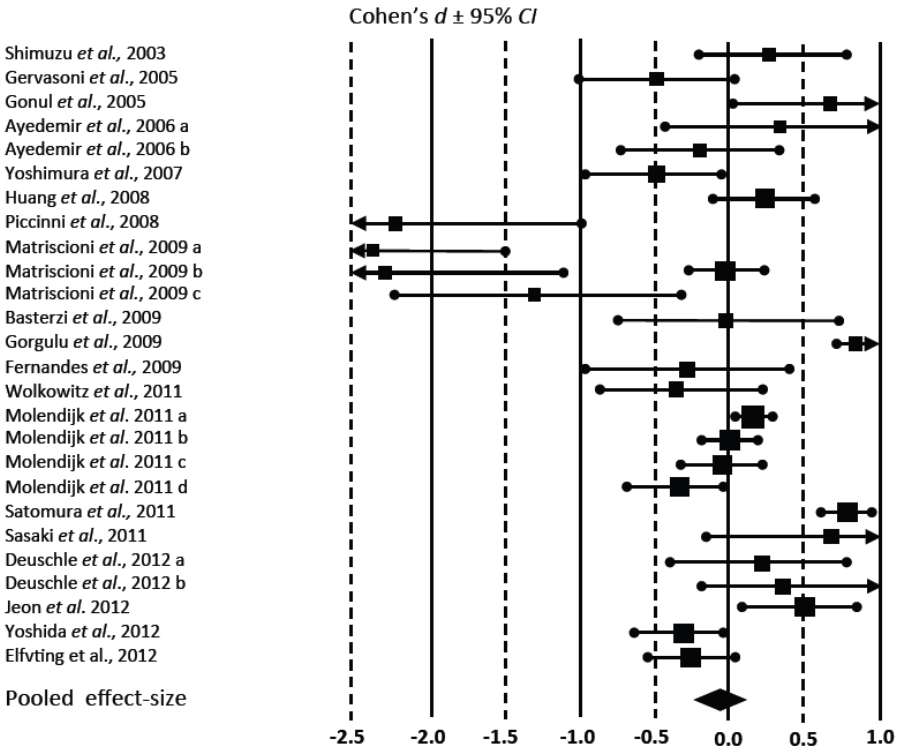

Figure S3. Forest plot for random effect meta-analysis on differences in serum BDNF concentrations between healthy controls and antidepressant-treated depressed patients. The sizes of the squares are proportional to sample size.

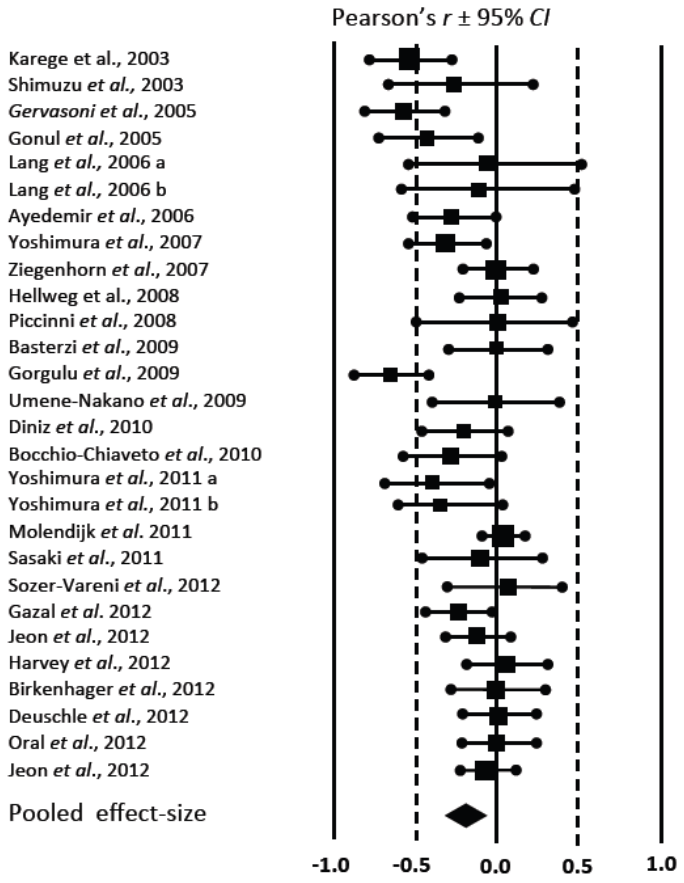

Figure S4. Forest plot for random effect meta-analysis on the continuous relation between serum BDNF concentrations in antidepressant-free depressed persons. The sizes of the squares are proportional to sample size.

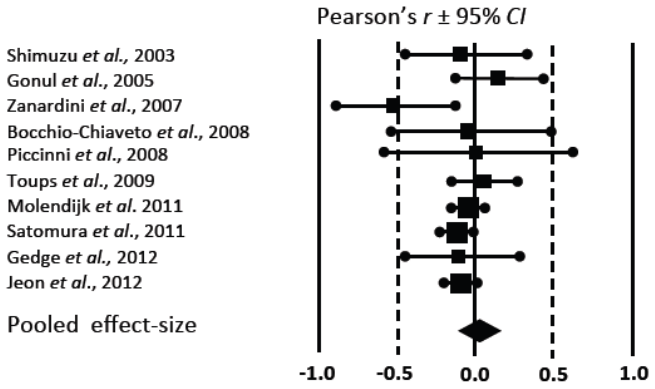

Figure S5. Forest plot for random effect meta-analysis on the continuous relation between serum BDNF concentrations in antidepressant-treated depressed persons. The sizes of the squares are proportional to sample size.

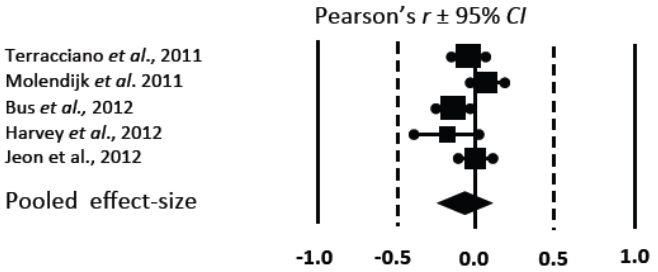

Figure S6. Forest plot for random effect meta-analysis on the continuous relation between serum BDNF concentrations in healthy control subjects. The sizes of the squares are proportional to sample size.

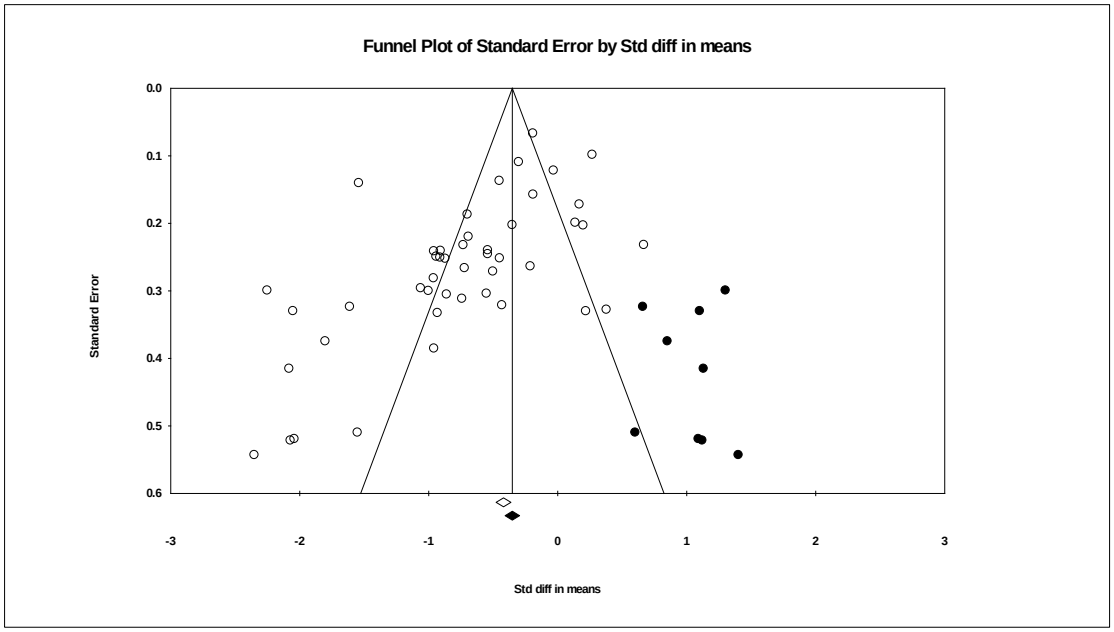

Figure S7. Funnel plot and trim-and-fill estimation showing the typical pattern of publication bias in the meta-analyses on differences in serum BDNF concentrations among healthy controls and antidepressant-free depressed patients. White data points depict observed associations and black data points imputed values. The white diamond depicts the aggregated point estimate (d = -0.71, 95% CI = -0.89 — -0.53, P < .00000001) and the black diamond the aggregated point estimate after the imputation of 10 studies (d = -0.47, 95% CI = -0.64—-0.27, P < .000001), resulting in a symmetrical funnel-plot.

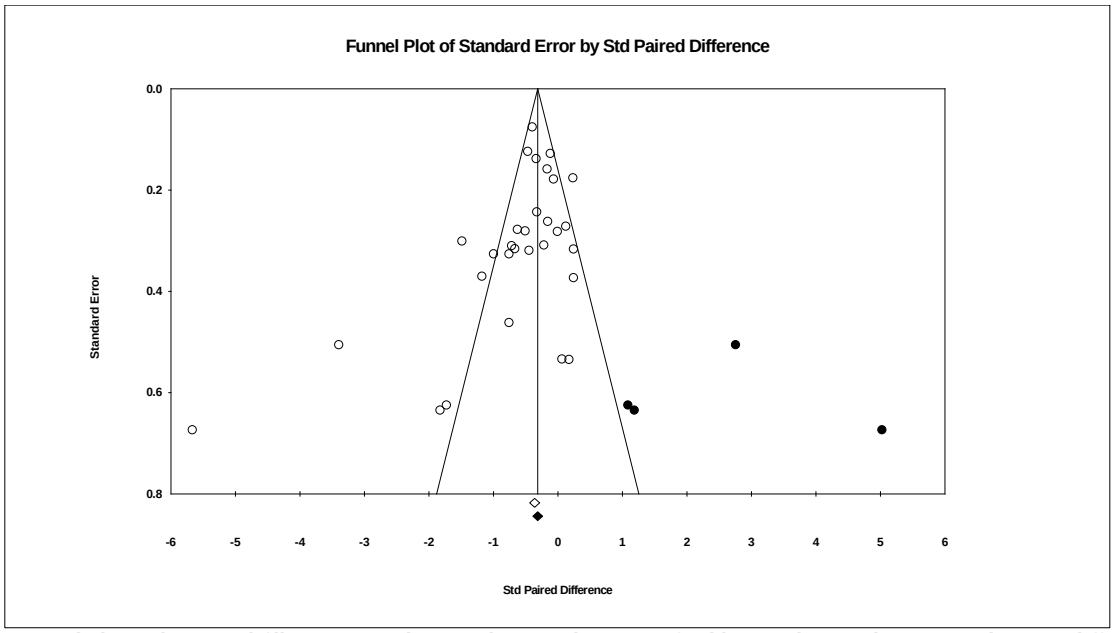

Figure S8. Funnel plot and trim-and-fill estimation showing the typical pattern of publication bias in the meta-analyses on differences in BDNF concentrations among antidepressant-free and treated depressed patients. White data points depict observed associations and black data points imputed values. The white diamond depicts the aggregated point estimate (d = -0.56, 95% CI = -0.77 — - 0.35, P < .000001) and the black diamond the aggregated point after the imputation of 4 studies (d = -0.34, 95% CI = -0.59 — - 0.09, P < .0001), resulting in a symmetrical funnel-plot.

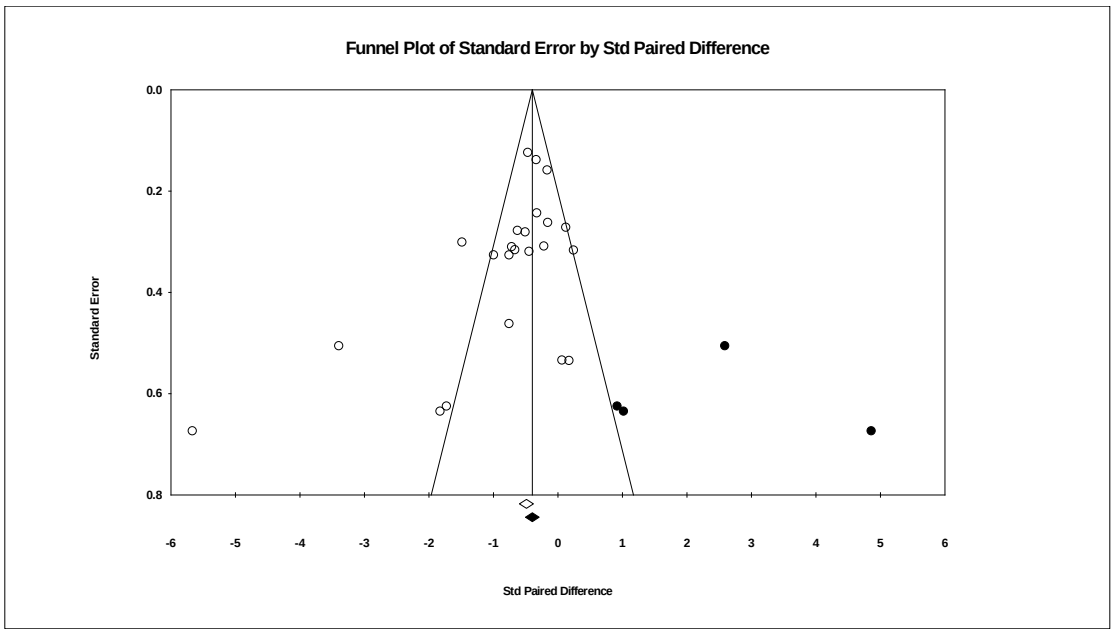

Figure S9. Funnel plot and trim-and-fill estimation showing the typical pattern of publication bias in the meta-analyses on differences in serum BDNF concentrations in treatment studies that reported on serum BDNF concentrations. White data points depict observed associations and black data points imputed values. The white diamond depicts the aggregated point estimate (d = -0.74, 95% CI = -1.04 — -0.45, P < .0000001) and the black diamond the aggregated point estimate after the imputation of 4 studies (d = -0.41, 95% CI = -0.76 — - 0.06, P < .001), resulting in a symmetrical funnel-plot.

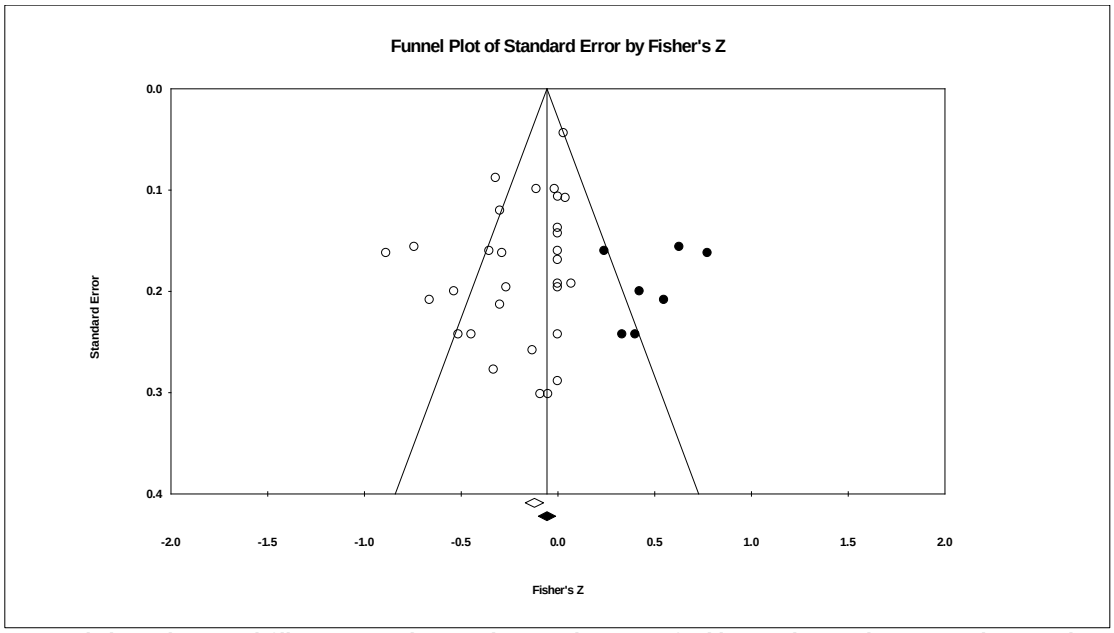

Figure S10. Funnel plot and trim-and-fill estimation showing the typical pattern of publication bias in the meta-analyses on the correlation between serum BDNF concentrations and the symptom severity of depression in antidepressant-free depressed patients. White data points depict observed associations and black data points imputed values. The white diamond depicts the aggregated point estimate (r = -0.19, 95% CI = -0.28 — - 0.10, P < .00001) and the black diamond the aggregated point estimate after the imputation of 7 studies (r = -0.08, 95% CI = -0.09 — 0.03, P = .42), resulting in a symmetrical funnel-plot.

APPENDIX IV

Chapter 9 A systematic review and meta-analysis on the association between BDNF val^{66}met and hippocampal volume – a genuine effect or a winners curse?

Table S1. Evaluation of the included records according to the Strenghtening the Reporting of Genetic Association Studies (Little *et al*., 2009) and Strenghtening Reporting of Observational Studies in Epidemiology (von Elme *et al*., 2007).

	STREGA and STROBE quality checklist items Y; meets the criterion, N; does not meet the criterion, NA; not applicable											
Author, year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Overall quality score**
Pezawas *et al*., 2004	Y	Y	Y	Y	Y	Y	NA	Y	N	NA	Y	7 Y, 2 N, 2 NA = 0.78
Szeszko *et al*., 2005	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	11 Y, 0 N, 0 NA = 1.00
Agartz *et al*., 2006	Y	Y	Y	Y	Y	Y	NA	N	N	Y	Y	8 Y, 2 N, 1 NA = 0.80
Bueller *et al*., 2006	Y	Y	Y	Y	N	Y	N	Y	Y	Y	N	8 Y, 3 N, 0 NA = 0.73
Frodl *et al*., 2007	Y	Y	Y	Y	Y	N	NA	Y	Y	Y	N	8 Y, 2 N, 1 NA = 0.80
Miyajima *et al*., 2008	Y	Y	Y	Y	Y	Y	NA	Y	Y	Y	NA	9 Y, 0 N, 2 NA = 1.00
Takahashi *et al*., 2008	Y	Y	Y	Y	Y	Y	NA	N	Y	Y	NA	9 Y, 1 N, 2 NA = 0.89
Chepenik *et al*., 2009	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	11 Y, 0 N, 0 NA = 1.00
Dutt *et al*., 2009	Y	Y	Y	Y	Y	Y	NA	N	N	Y	NA	7 Y, 2 N, 2 NA = 0.78
Gatt *et al*., 2009	Y	Y	Y	Y	Y	Y	NA	N	N	Y	NA	7 Y, 2 N, 2 NA = 0.78
Jessen *et al*., 2009	Y	N	Y	Y	N	Y	NA	N	N	Y	NA	5 Y, 4 N, 2 NA = 0.56
Joffe *et al*., 2009	Y	Y	Y	Y	Y	Y	NA	Y	Y	Y	NA	9 Y, 0 N, 2 NA = 1.00
Schofield *et al*., 2009	Y	N	Y	Y	Y	N	NA	Y	Y	Y	NA	7 Y, 2 N, 2 NA = 0.78
Toro *et al*., 2009	Y	Y	Y	Y	Y	Y	NA	Y	Y	Y	NA	9 Y, 0 N, 2 NA = 1.00
Benjamin *et al*., 2010	Y	N	Y	Y	N	Y	NA	N	N	Y	NA	5 Y, 4 N, 2 NA = 0.56
Karnik *et al*., 2010	Y	Y	Y	Y	N	Y	N	N	Y	Y	N	7 Y, 4 N, 0 NA = 0.64
Koolschijn *et al*., 2010	Y	Y	Y	Y	Y	Y	NA	Y	Y	Y	NA	9 Y, 0 N, 2 NA = 1.00
Cole *et al*., 2011	Y	Y	Y	Y	Y	Y	NA	Y	Y	Y	NA	9 Y, 0 N, 2 NA = 1.00
Gerritsen *et al*., 2011	Y	Y	Y	Y	Y	Y	NA	N	Y	Y	NA	8 Y, 1 N, 2 NA = 0.89
Gonul *et al*., 2011	Y	Y	Y	Y	N	Y	NA	Y	Y	Y	NA	8 Y, 1 N, 2 NA = 0.89
Gruber *et al*., 2011	Y	Y	Y	Y	Y	Y	NA	N	Y	Y	NA	8 Y, 1 N, 2 NA = 0.89
Kanellopoulos *et al*., 2011	Y	Y	Y	Y	Y	Y	NA	N	Y	Y	NA	8 Y, 1 N, 2 NA = 0.89
Richter *et al*., 2011	Y	Y	Y	Y	Y	Y	NA	Y	Y	Y	NA	9 Y, 0 N, 2 NA = 1.00
Milan Sanchez *et al.,* 2012	Y	Y	Y	Y	Y	Y	NA	N	Y	Y	NA	8 Y, 1 N, 2 NA = 0.89
Molendijk *et al.,* 2012	Y	Y	Y	Y	Y	Y	NA	Y	Y	Y	NA	9 Y, 0 N, 2 NA = 1.00

Criteria were assessed independently by 2 of the authors. Inconsistencies were evaluated in consensus meetings. Agreement among the raters proved to be excellent with Cohen's Kappa = 0.83, standard error = 0.04. Please contact the corresponding author for information about the actual reason for scoring a yes, a no, or a not applicable

STREGA (Little *et al*., 2009) and STROBE (von Elme *et al*., 2007) criteria: (**1**) Clear statement of the objectives and the hypothesis (**2**) Clear eligibility criteria for study participants (**3**) Clear definition of all variables (**4**) Replicability of Statistical methods (**5**) Assessment of Hardy-Weinberg equilibrium (**6**) Assessment of ethnicity (**7**) Addressing the problem of mixed ethnicities (**8**) Sufficient descriptive data (**9**) Statement of genotype frequencies (**10**) Sample in Hardy-Weinberg equilibrium (**11**) Consideration of population stratification (if applicable)

APPENDIX V

Chapter 10 General discussion: The poll

Two know more than one: a poll

The conventional option to prove association is observation and experimentation. However, and notwithstanding the fact that some experiments have been performed on the relation between peripheral and central BDNF functioning, the literature on this topic appears confusing to me. Therefore, I chose to run a poll among experts/researchers from around the world.

Through PUBMED I identified the 50 most recently published papers that had *BDNF* and *depression* in their title (regardless whether it were studies on humans, rats,). From each paper I extracted the email address of the corresponding author and I wrote an email to him/her. I asked the following question: *What do you think, how relevant are serum BDNF concentrations with regard to (human) depression*? I prompted them to give a short answer by adding answer options: (A) *relevant, will add to our knowledge on depression*, (B) *irrelevant*, (C) *don't know yet, but the future probably will tell* or (D) *I do not believe in a biological basis of depression whatsoever*. I added that a short explanation could be given but that this was not necessary for my current concerns. While doing this I reckoned that a poll like this could yield biased results because all the persons work on the *topic* and therefore their responses could well be overly positive. To overcome this at least a little I set out a second poll. I identified the 50 most recently published papers that had *cognitive-* or *interpersonal theory* and *depression* in their title (again regardless their exact topic). I asked the corresponding authors of these papers the same question as I asked the BDNF oriented authors and I provided them with the same answer categories. A full version of the e-mail and the list of authors that was contacted can be found below.

Seventy-two percent (*n* = 36) of the BDNF oriented researchers and 48 percent (*n* = 24) of the cognitive/interpersonal-oriented researchers responded to my request. This difference in response rate was statistically significant (χ^2_1 = 6.01, *P* = 0.01). Of the BDNF oriented authors who responded, 56 percent (*n* = 26) agreed with the proposition that peripheral BDNF concentrations are relevant parameters for depression, 6 percent (*n* = 3) disagreed, and 36 percent (*n* = 13) suggested that for now there is too little knowledge on the topic to come to conclusions. The interpersonal/cognitive-oriented authors who responded to the poll were somewhat more pessimistic. In this group, 25 percent (*n* = 6) agreed with the proposition, 25 percent (*n* = 6) disagreed and 50 percent (*n* = 12) suggested that the future probably would inform us. The pattern of responses between the two groups of authors differed significantly (χ^2_2 = 6.44, *P* = 0.04) such that more of the BDNF oriented authors agreed with the proposition that peripheral BDNF concentrations are relevant parameters for depression. The response frequencies of both polls are provided in the **Table ↓**.

Table. Results from the poll by research orientation

	Biologically oriented colleagues			Cognitive oriented colleagues		
	n = 50	% *n* [1]	% *responders* [2]	*n* = 50	% *n* [1]	% *responders* [2]
A - *agree*	20	40%	56%	6	12%	25%
B - *disagree*	3	6%	9%	6	12%	25%
C - *future will tell*	13	26%	36%	12	24%	50%
D - *non-believer*	0	0%	0%	0	0%	0%
No response	14	28%	*NA*	26	52%	*NA*

Abbreviations: NA; Not Applicable
[1] Percentage of the *n* = 50 to which I send out the poll
[2] Percentage of the persons that actually responded on the poll

The main lesson to learn from this poll is that the large majority of researchers either agrees (43 percent) with the proposition that serum BDNF concentrations are relevant with regard to depression *or* expresses the belief that the future will inform us on this issue (42 percent). Only 15 percent explicitly disagrees with the notion that serum BDNF concentrations are relevant with regard to

depression. In this sense, the poll was helpful in that most authors see either relevance in the use of serum BDNF concentrations as parameters for depression or suggests that more research will bring definite answers. Tentatively, this strengthens the belief that serum BDNF concentrations are relevant with regard to depression – but again, a systematic exploration would suit the question better and therefore is be very welcome.

The e-mail that was sent out

Topic: Question: BDNF in the periphery, how relevant is that for central processes?

Dear Dr. *name corresponding author, dear colleague,*

I have a question for you. I'm writing my PhD thesis on serum BDNF concentrations – a topic related to your research interests (attached you can find one of our papers on serum BDNF levels in depressed persons). I'm in the middle of wrapping it all together and writing the final thesis discussion. Already for a while I noticed debate in the literature on the use of peripheral BDNF levels as a reliable mirror of neurotrophic functioning in the central nervous system. For my thesis discussion I wanted to know how other scientists, who work in related fields [but not necessarily the exact same], think about this issue. So, I decided to send out a poll to the corresponding authors of the 50 most recent papers that have BDNF in their title (regardless the precise topic) to learn about the opinion of the authors on this topic. You happen to be in that group with your paper in *the Journal in which the paper is published*.

My question to you is: *What do you think, how relevant are serum BDNF concentrations with regard to (human) depression*?

The corresponding letter A, B, C, or D is enough for me as response
A '*Relevant, will ad to our knowledge on depression and neuronal plasticity in the brain*'
B '*Irrelevant, won't add a lot to our knowledge on depression and neuronal plasticity in the brain*'
C '*Don't know, maybe the future will tell*'
D *'I do not believe in a biological basis of depression whatsoever*'

You can add a short explanation if you wish. I'm interested in that but it is not necessary for my current purpose.

Thank you in advance for your response, all the best,

Marc Molendijk

Note. Attached you can find one of our papers on serum BDNF concentrations in depressed persons that has been published in *Molecular Psychiatry*.

Authors in the poll -- authors who responded are underlined

BDNF oriented authors
C Duarte (Portugal), M Miquel (Spain), K Felmingham (New Zealand), K Iqbal (USA), H Scharfman (USA), D Jon (Korea), M Fawzi (Egypt), M Shamsul Ola (Saudi Arabia), S Vivekanandhan (India), G Morton (USA), X Zhang (China), F Lotrich (USA), N Perroud (Switzerland), L Ricceri (Italy), J Luykx (the Netherlands), R Ting-A-Kee (Canada), G Hasler (Switzerland), R Rodríguez-López (Spain), E Ottem (USA), L-M Wu (China), N Mechawar (Canada), K Ressler (USA), X Xiayixiayi (China), M Soleimani (Iran), S Cramer (USA), D Carlino (Italy), D Srivastava (UK), J-M Kim (Korea), G Réus (Brazil), T Sakharnova (Russia), E McNay (USA), Y Hung (Taiwan), J Charoenphandhu (Thailand), K Kauppi (Sweden), D Carbone (USA), M Gilbert (USA), D Ron (USA), M Dmitrzak-Weglarz (Poland), J Yang (USA), W Umene-Nakano (Japan), S Miller (USA), J-H Chae (Korea), V Stelzhammer (UK), Z-Y Chen (China), F Fumagalli (Italy), R Dalle Molle (Brazil), N Cardoner (Spain), T Endres (Germany), C Ernst (Canada), Y Tizabi (USA), E Tongiorgi, (Italy).

Cognitive/inter-personal oriented authors
P Pössel (USA), F Jollant (Canada), J Johnstone (New Zealand), Melitta Fischer-Kern (UK), P Thoma (Germany), M Constantino (USA), H O'Mahen (UK), M van Hees (the Netherlands), J Ogrodniczuk (Canada), M Hochberg (USA), R Auerbach (USA), S Winkeljohn Black (USA), E Sheets (USA), F Peters (the Netherlands), M Flynn (USA), K Mclaughln (USA), N Wongpakaran (Thailand), B D'Antono (Canada), J Ehrenreich-May (USA), M Constantino (USA), G Stein (USA), A Forsman (Finland), M Power (Scotland), F Renner (the Netherlands), J Stewart (Canada), T Kühnen (Germany), T Berger (Switserland), J Jakobsen (Danmark), J McCullough (USA), G Tasca (Canada), L Hides (Australia), L Lemmens (the Netherlands), J Goodman (USA), M Serfaty (UK), F Doyle (Ireland), M McKinnon (Canada), L Sockol (USA), S Rueger (USA), D Klein (USA), L Rood (the Netherlands), L Wolkenstein (Germany), K Iverson (USA), G Pomaki (Canada), R Trivedi (USA), Pauline Slade (UK), H Teunissen (the Netherlands), C Beevers (USA), S Hollon (USA), E Schramm (Germany), D Dozois (Canada).