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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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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-BDNF 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 1** details the intra-cellular cascades that follow after the binding of BDNF with TrkB.

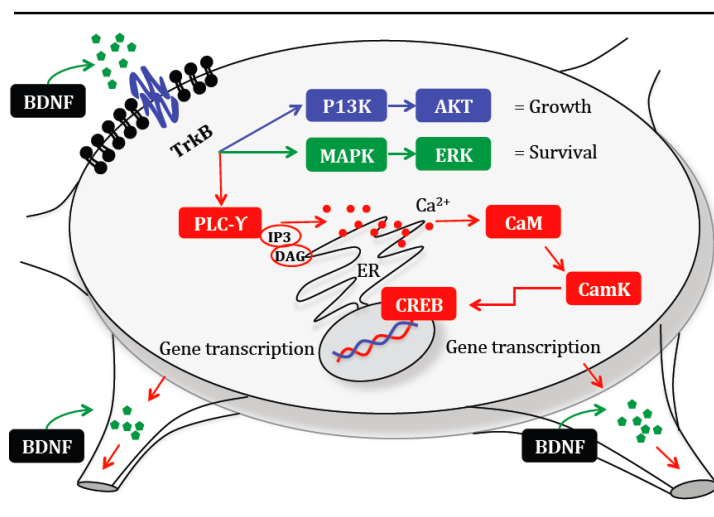


Figure 1. Overview of the signaling cascades that follow TrkB activation: (I) P13K/AKT (regulates translation initiation and neuronal survival); (II) MAPK (CREB phosphorylation); (III) PLC-γ (CREB phosphorylation).

Abbreviations:

BDNF, Brain-Derived Neurotrophic Factor
Ca²⁺, 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 (<i>e.g.</i> , 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 (<i>e.g.</i> , 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)
IIIX	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; Bunney 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 rationale 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 ↓**).

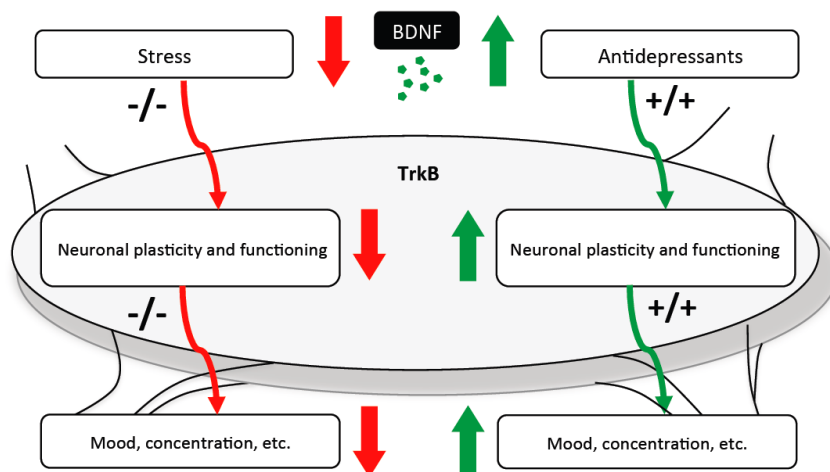


Figure 2. A schematic representation of the neurotrophin hypothesis

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, Shimizu *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 val⁶⁶met (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 val⁶⁶met 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 Szeszko *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 val⁶⁶met 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 val⁶⁶met) as parameters or proxies for neurotrophic functioning in the brain. For an overview of the breakthroughs in the research into the neurotrophin 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 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 val⁶⁶met 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 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 val⁶⁶met 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). Notwithstanding 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 val⁶⁶met 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 val⁶⁶met 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 val⁶⁶met 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.

