The Role of Brain-Derived Neurotrophic Factor (BDNF) in Stress-Related Brain Disorders

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Summary

It is well accepted that we live in a modern industrialized environment that pushes the limit of our physiology and restricts our body to respond to additional stressors. This leads to a shifted vulnerability or resilience, depending on the individual genetic background and epigenetic factors, to the effects of stress which might result in the development of stress-related brain disorders like major depression. Understanding the cellular and molecular bases of stress-related mental disorders is crucial in the effort to develop new treatments, since treatment outcomes have improved only slightly in the past few decades. Only approximately 50% of patients with major depressive disorder show response to treatment after one treatment trial. The long duration required concluding treatment success or failure is a difficult and frustrating experience for the patient. Therefore, a current goal is moving towards the field of personalized medicine and biomarker research using patient specific profiles, with the perspective of providing more effective treatment.

Evidence has been raised demonstrating the complex outcome of stress on the BDNF system and that the protein is a critical backbone in the functioning and well-being of the central nervous system. The protein is originally derived from the brain and related to neurotrophic actions which promote cell survival and development in the brain. Additionally, it is found in the periphery where it is stored in blood platelets and can be released into the serum. Several studies support the "neurotrophin hypothesis of depression", which postulates that reduced brain levels of BDNF could contribute to atrophy and cell loss as observed in the hippocampus of depressed subjects. Until now several studies have demonstrated that stress causes impaired neurogenesis in brain structures, and that BDNF down-regulation is one of the hallmark events that occur. However, the precise mechanism underlying this down-regulation has not been fully understood. Stress *per se* might not be sufficient to cause a psychiatric disorder like depression. It is believed that interactions between a genetic predisposition and environmental factors play a major role in the development of stress-related brain diseases. Furthermore, the BDNF regulation system seems to be very complex because of several influencing factors. Given that BDNF expression is decreased by stress and related to mood disorders, increased by antidepressants, and normalized in patients taking antidepressants, many investigators have focused on BDNF as a "biomarker" and potential target for treatment in major depression.

The purpose of the present study was to elucidate the role of BDNF in stress related-brain disorders regarding the interplay of stress with the human homeostasis connected to sleep and prediction of therapy outcome. Therefore, we assessed systemic serum BDNF levels from human subjects. We could reveal that (1) there is a connection between sleep and serum BDNF levels, (2) sleep mediates the relationship between stress and BDNF and (3) a diurnal variation of serum BDNF levels is linked to favourable antidepressant treatment response.

(1) Sleep problems are common features in many stress-related mental disorders, problems that may lead to impairment of physical and mental health because sleep loss is often followed by higher stress vulnerability. Thus, insomnia is very common among depressed patients. Although a majority of studies have concentrated on specifying the role of BDNF in depression, the relation between BDNF and insomnia has not been a focus of recent research. Therefore, we investigated serum BDNF levels of subjects with current symptoms of insomnia and non-sleep disturbed controls including patients. We found subjective sleep impairment to be associated with lower serum BDNF levels, whereas reported good sleep was related to higher serum BDNF levels, as shown for those suffering from current insomnia compared with sleep-healthy subjects. Furthermore, serum BDNF levels were correlated with severity of insomnia in all participants. To confirm the relevance of this finding, we investigated an additional control sample recovered from occupational burnout after 12 weeks of aerobic exercise training. Again, serum BDNF levels were significantly lower in those reporting symptoms of fatigue compared with sleep-healthy subjects and were correlated with symptoms of tiredness and fatigue known to reflect malfunction of sleep. Hence, we suggest that serum BDNF levels are not associated with a specific diagnosis, but may be associated with insomnia symptoms independent of diagnosis. These results consolidate the awareness that when serum BDNF levels are analysed, insomnia symptoms should be carefully controlled, as well as improvements in sleep during therapy interventions in stress-related mood disorders.

(2) However, the underlying mechanism in this relationship between sleep and BDNF has to be further elucidated. It might be possible that sleep impairment reflects a chronic stressor influencing the brain and in turn is accompanied by a deregulation of the HPA system, leading in the long term to decreased BDNF levels. Consequently, we wanted to reveal how stress and sleep could affect serum BDNF levels. Therefore we reanalysed the previous study were we could already demonstrate an association between decreased serum BDNF levels and insomnia severity (see [1]), by including further data. Remarkably, we could demonstrate an interaction between stress and insomnia with an impact on serum BDNF levels. With regard to insomnia severity, we divided all participants into three subgroups reflecting their score on the *Insomnia severity Index*: subjects with no insomnia, sub-threshold insomnia and clinical insomnia. Insomnia severity groups and stress each exhibited a significant main effect on serum BDNF levels. Furthermore, insomnia severity was associated with increased stress experience affecting serum BDNF levels. Notably, the association between stress and BDNF was only observed in subjects without insomnia. Searching for an explanation in the interplay between stress, sleep and BDNF we used a mediation model, which identified sleep as a mediator of the association between stress and serum BDNF levels. Here we could show for the first time that the interplay between stress and sleep impacts BDNF levels suggesting an important role of this relationship in the pathology of stress-related brain disorders. These basic findings support the role for sleep as key mediator at the connection between stress and BDNF. We propose the hypothesis that whether sleep is

maintained or disturbed might explain why some individuals are able to handle a certain stress load while others develop a mental disorder.

(3) Finally, we complemented our previous work by investigating serum BDNF levels within a therapeutic intervention setting focussing on the association between BDNF and depressive symptoms as well as prediction of treatment response. Patients suffering from major depressive disorder, naïve to sleep therapy experienced a partial sleep deprivation (PSD) supplementary to an on-going mono-therapy with mirtazapine. For serum sampling blood was obtained at seven different time points: at 8am (t1), 2pm (t2) and 8pm (t3) for baseline (day 0), at 1.30am (t4) during PSD, as well as 8am (t5), 2pm (t6) and 8pm (t7) after PSD (day 1).We could show that serum BDNF levels followed a diurnal pattern during the day before therapy intervention at baseline with high levels peaking in the morning and decreasing throughout the day. This diurnal pattern on the day before PSD was associated with an acute antidepressant treatment response since diurnal variation in serum BDNF was absent in non-responders. Responders of the day after PSD revealed significantly increased serum BDNF levels in combination with a prominent diurnal variation of BDNF levels at baseline before PSD compared to non-responders. Notably, the same was also relevant for long-term responders, who showed an improvement of depressive symptoms after two weeks of on-going treatment. Again, day 14 non-responders did not show this diurnal variation of BDNF levels. BDNF levels maintained at the same low level throughout the day, resulting in a flat line. In addition, serum BDNF levels were increased for acute and long-term responders at the day after PSD when compared to non-responders. This increase in BDNF levels on the day after PSD was correlated with improved mood and relaxation after a recovery night. In addition, the improvement of depressive symptoms after two weeks of on-going treatment was correlated with an increase of serum BDNF levels in all patients. Hence, our results indicate that the elasticity in diurnal serum BDNF variation is associated with favourable treatment response to PSD in patients suffering from MDD. Therefore, a normalized BDNF serum profile which oscillates in a circadian fashion seemed to precede, rather than follow a favourable treatment outcome in depressed patients. Thus, we suggest that diurnal profiling of BDNF should be monitored at baseline especially before therapeutic intervention starts for the purpose of early response prediction.

In summary, our work demonstrates that sleep is associated with serum BDNF levels. This interplay is also influenced by stress and we could show that sleep is a mediator in the relationship between stress and BDNF. Therefore sleep, stress and BDNF seem to accomplish an important relationship in the pathology of stress-related brain disorders. Furthermore, we show that a diurnal variation of serum BDNF levels during the day before therapy intervention is associated with antidepressive response. This diurnal variation might allow the prediction for becoming responder or non-responder to a given antidepressive therapy. Notably, our results support the awareness of assessing sleep and sleep

improvement next to a diurnal variability profile, when BDNF levels are analysed, to promote antidepressive therapy and individual, personalized treatment.

A. Introduction

The brain and body continuously adapt. In recent years the responsiveness of the price that body and brain have to pay in a '24/7' society have been grown, where social and physical environment have an enormous impact on physical and mental well-being (McEwen and Gianaros 2010). This modern industrialized society pushes the limit of our physiology and in turn restricts the capacity of the brain and body to respond to additional stressors. As a result, the brain is rendered to be more vulnerable or resilient, depending on an individual genetic background and epigenetic factors, to the effects of stress. Observing pathological alterations of brain structure and function remains markedly more difficult (Krishnan and Nestler 2008) than compared to other organs. Even with a long history of research in this field, the control of mood is still not solved and the development of efficient drugs is far from being satisfactory (Rush and Thase 1997; Kelly and Leonard 1999; Ioannidis 2008). Until now, studies on humans rely on occasions in which certain brain structures are absent – accidently or not – or on *post-mortem* tissues. Most diagnosis of brain disorders like depression, schizophrenia, anxiety or other stress-related alterations, in common named stress-related mood disorders, are subjective and rely on the documentation of a number of symptoms that significantly impair brain functioning for certain duration. This symptom-based diagnostic approach is accompanied by evident difficulties since diagnostic criteria might overlap with different conditions and disease patterns, since co-morbidity is quite common. Therefore, neuropsychiatric brain disorders are the most disabling of all medical disorders. According to statistics from community studies in European Union (EU) countries, Iceland, Norway and Switzerland 27% of the adult population (18-65 years) have been experienced at least one of a series of mood disorders in the past year, with an estimation of 83 million people being affected (WHO, 2013). Mood disorders frequently appear in life, run a chronic course and adversely affect the prognosis of other medical illnesses (Charney and Manji 2004). In the WHO European Region mental health problems affect one in four people at some time in life. Each year, 25% of the population suffer from depression or anxiety disorder and about 50% of major depressions are untreated. About 123.800 people commit suicide with a mood disorder background every year. The costs of mood disorders in the EU are about 170 EUR billion per year (WHO, 2013).

Understanding the cellular and molecular bases of these disorders is crucial in the effort to develop new treatments. Despite progress in understanding the neurobiology of brain disorders, treatment outcomes have improved only slightly in the past few decades even if broadening the target spectrum of pharmaceuticals, especially antidepressants. Only approximately 50% of patients with major depressive disorder (MDD) show response to treatment after one treatment trial, and only 30% of patients reach full remission (Weizman, Gonda et al. 2012). There is a significant decrease in the remission rate after four treatment trials (Rush, Trivedi et al. 2006). The long duration required concluding treatment success or failure (eight to twelve weeks) is a difficult and frustrating experience

for the patient (Sadock 2007). Besides failure to reach remission, the relapse rate is over 40%, especially in patients who did not achieve full remission (Zisook, Ganadjian et al. 2008; Sinyor, Schaffer et al. 2010). Therefore, a current goal is moving towards the field of personalized medicine and biomarker research with patient-specific profiles incorporating genetic and genomic data, as well as clinical and environmental factors, with the perspective of providing more effective treatment individually designed to a given patient or sub-population (Crisafulli, Fabbri et al. 2011; Porcelli 2011).

A.1 Neurotrophins

Neurotrophins are considered to play a pivotal role in various aspects of neural function including survival, development, function, and activity-dependent synaptic plasticity. The influence of neurotrophins enfolds developmental neurobiology to neurodegenerative, and psychiatric disorders. As first neurotrophin, nerve growth factor (NGF) was identified, which was found during a search for such survival factors (Levi-Montalcini 1966).

The surprising discovery that neurotrophins and their receptors do not exist in *Drosophila melanogaster* or *Caenorhabditis elegans* reinforce the idea that these proteins are not absolutely necessary for the development of neuronal circuits *per se*, but are involved in somehow higher-order activities (Chao 2003). In the mammalian brain, four neurotrophins have been identified: NGF, brain-derived neurotrophic factor (BDNF) (Barde, Edgar et al. 1982), neurotrophin-3 (NT-3), and neurotropin-4 (NT4) (Hohn, Leibrock et al. 1990). All of these are considered to originate from a common ancestral gene, exhibit similarities in sequence and structure, and are therefore collectively named neurotrophins (Huang and Reichardt 2001). These closely related, highly basic proteins act by binding to two distinct classes of transmembrane receptors: the p75 neurotrophin receptor ($p75^{NTR}$), a member of the tumour necrosis factor (TNF) receptor superfamily (Chao 2003), and the family of tropomyosin-related receptor tyrosine kinases (Trks), which include TrkA, TrkB and TrkC (Kaplan and Miller 2000; Dechant and Barde 2002; Chao 2003; Huang and Reichardt 2003). Like other secreted proteins, neurotrophins arise from precursors, pro-neurotrophins (30-35kDa), which are proteolytically cleaved to produce mature proteins (12-13kDa) (Seidah, Benjannet et al. 1996). Pro-neurotrophins bind with high affinity to $p75^{NTR}$, which for years was considered to be a low-affinity receptor. Of note, all neurotrophins bind to $p75^{NTR}$ with a very similar affinity (Rodriguez-Tebar, Dechant et al. 1991) but mature neurotrophins selectively interact with their individual high-affinity protein kinase receptors. This interaction leads to cell survival, whereas binding of pro-neurotrophins to $p75^{NTR}$ is involved in apoptosis (Lu, Pang et al. 2005).

A.2 BDNF cell biology

BDNF and related family members influence the proliferation, differentiation, and growth of neurons during development, but are also expressed in the adult brain and play a critical role in the survival and function of mature neurons (McAllister 2002). The protein is moderately sized and charged with an isoelectric point about $I_p \approx 10.1$, which indicates the strong basicity (Barde, Edgar et al. 1982). Furthermore it is characterised by a high specific activity of 0.4ng/ml per unit (1 unit defines the protein concentration in ng/ml, where 50% of neurons survive in cell culture) or rather $3x10^{11}$M (Barde, Edgar et al. 1982). BDNF is expressed at high levels in limbic brain structures implicated in mood disorders, including the hippocampus, prefrontal cortex (PFC), and amygdala. Cellular actions of BDNF are mediated through Trk receptor type B and $p75^{NTR}$. The $p75^{NTR}$ was shown to transmit signals important for determining which neurons survive during development. Functional, mature BDNF is a polypeptide of 119 amino acids, about 14kDa in size, forms stable, non-covalent dimers (28kDa) (Barde, Edgar et al. 1982; Mowla, Farhadi et al. 2001; Lessmann, Gottmann et al. 2003) and has been shown to directly bind and dimerize TrkB receptors stimulating autophosphorylation of specific tyrosine residues present in their cytoplasmic kinase domains, present on the cellular membrane of receptive cells in the central nervous system (Greenberg, Xu et al. 2009).

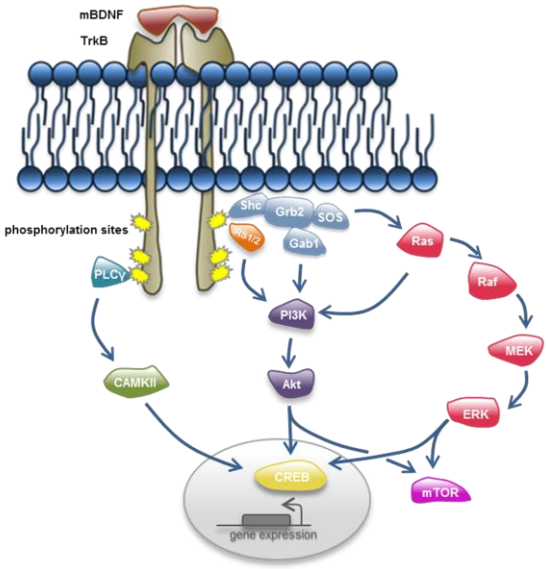

Figure 1: BDNF signalling pathways. Mature BDNF forms a dimer and binds TrkB with high affinity to induce its dimerization and autophosphorylation of tyrosine residues in the cytoplasmic kinase domain. These residues serve as docking sites for effector molecules and trigger the activation of three main signalling pathways: PLCγ, PI3K and ERK cascades. These lead to phosphorylation and activation of the transcription factor CREB mediating transcription of genes essential for the survival and differentiation of neurons. The recruitment of PLCγ increases intracellular Ca^{2+} levels and leads to activation of CaMKII to phosphorylate CREB. PI3K can be activated via the Shc/Grb2/SOS complex through Gab1 and by IRS1/2. Lipid products generated by the activated PI3K, the phosphatidylinositides bind and activate protein kinase Akt, upstream of CREB. The ERK cascade can be activated both by Shc/Grb/SOS complex and by PI3K. ERK phosphorylation leads directly to CREB phosphorylation. **PLCγ** – phospholipase Cγ, **PI3K** – phosphatidylinositol 3-kinase, **ERK** – extracellular signal-regulated kinase, **CaMKII** – calcium-calmodulin dependent kinase, **Shc** – src homolgy domain containing, **Grb2** – growth factor receptor-bound protein 2, **SOS** – son of sevenless, **Gab1** – Grb-associated binder 1, **IRS1/2** – insulin receptor substrates 1/2, **CREB** – cAMP-calcium response element bidning protein, **Ras** – GRP binding protein, **Raf** – Ras associated factor, **MEK** – MAP/Erk kinase (adapted from Cunha (Cunha, Brambilla et al. 2010)).

This results in activation in one of three major intracellular signalling cascades: the mitogen-activated protein kinase (MAPK, or extracellular signal related kinase ERK) which activates several downstream effectors; the phosphatidylinositol-3 kinase (PI3K) which activates serine/threonine kinase AKT; and the phospholipase-C- γ (PLCγ) pathway which leads to activation of protein kinase C (Tanis, Newton et al. 2007) (Figure 1). BDNF is a glycoprotein and secreted in response to neuronal activity, largely via the regulated pathway and derived from both pre-and postsynaptic sites (Waterhouse and Xu 2009). The action of BDNF signalling on synapses arises within seconds of stimulation or application/release of the factor (Kovalchuk, Holthoff et al. 2004) and results in sustained TrkB activation. In brief, rapid synaptic and ion channel effects are thought to depend on PLCγ-mediated release of intracellular calcium stores, and longer-lasting effects involving transcription are considered to be downstream of PI3K and MAPK pathways (Autry and Monteggia 2012). These cascades have been linked to neuroprotective effects of BDNF, as well as regulation of cell proliferation, differentiation, and survival (McAllister 2002).

Next to the central nervous system BDNF is also found in the periphery and therefore has been of particular interest, because of its potential role in non-neuronal tissues. BDNF mRNA has been found for example in the rat aorta (Scarisbrick, Jones et al. 1993), kidney, submandibular gland, ovary, dorsal root ganglia (Ernfors, Wetmore et al. 1990), heart (Hiltunen, Arumae et al. 1996), retina, muscle, lung (Maisonpierre, Belluscio et al. 1990; Maisonpierre, Le Beau et al. 1991), T and B immune cells (Kerschensteiner, Gallmeier et al. 1999), endothelial cells and platelets (Yamamoto and Gurney 1990). The TrkB receptor is present in peripheral targets including vascular endothelial cells (Donovan, Lin et al. 2000), vascular smooth muscle (Nemoto, Fukamachi et al. 1998), dorsal root ganglia neurons (McMahon, Armanini et al. 1994), Schwann cells (Alderson, Curtis et al. 2000), B (D'Onofrio, de Grazia et al. 2000) and T (Maroder, Bellavia et al. 1996) lymphocytes, and endocrine cells (Esteban, Hannestad et al. 1995). It has been shown that activated human T and B cells, and monocytes (Kerschensteiner, Gallmeier et al. 1999) produce BDNF and express truncated (Besser and Wank 1999) as well as full-length (Maroder, Bellavia et al. 1996; D'Onofrio, de Grazia et al. 2000) TrkB receptors on their cell surface.

The existence and function of BDNF in blood, particularly serum and plasma of humans and other mammals are poorly understood (Fujimura, Altar et al. 2002; Mori, Shimizu et al. 2003). Blood platelets are the major storage side of BDNF in blood, from which it can be released into the plasma through activation or clotting processes (Fujimura, Altar et al. 2002). After agonist stimulation during blood coagulation, roughly half of the BDNF contained in platelets is released at the site of injury (Burnouf, Kuo et al. 2012). The contribution of alternative sources of blood BDNF like endothelial cells (Nakahashi, Fujimura et al. 2000) and lymphocytes is believed to be marginal compared with the bulk release from platelets. Extracts from platelets contain 50 to 100 times the BDNF biologic activity of brain extract (Yamamoto and Gurney 1990). Human serum contains BDNF at far greater concentrations, which is about 100-fold higher (approximately 10-27ng/ml) compared to human

plasma (Rosenfeld, Zeni et al. 1995; Radka, Holst et al. 1996; Fujimura, Altar et al. 2002; Burnouf, Kuo et al. 2012) due to the fact that in serum a total, including the amount of BDNF released from the platelets, is detected. It has been suggested that almost all of the BDNF in serum originates from platelets and that freely circulating BDNF in blood binds to the surface of platelets, which could promote the internalization of BDNF through as yet unidentified binding sites that appear to be distinct from TrkB or p75 receptors (Fujimura, Altar et al. 2002). It is also suggested that platelets may serve as a reservoir for circulating BDNF (Fujimura, Altar et al. 2002). The absence of nuclei and lack of platelet protein synthesis is consistent with a dependence upon circulating BDNF. Furthermore, it was shown in a cell culture model that BDNF in platelets does not originate from megakaryocyte precursor cells of mature platelets (Fujimura, Altar et al. 2002). It is more likely, that the presence of BDNF in platelets results from both synthesis by vascular endothelial cells (Nakahashi, Fujimura et al. 2000) and internalization from blood circulation (Fujimura, Altar et al. 2002), rather than from *in situ* platelet synthesis because they contain only small to no amounts of BDNF mRNA probably derived from the cytoplasm of megakaryocytic (Yamamoto and Gurney 1990) cells, precursors of platelets, and megakaryocytes are believed to actually not contain BDNF (Fujimura, Altar et al. 2002). The reason why platelets are the major storage side of peripheral blood BDNF is not clear. A hypothesis, which has been suggested, deals with the assumption that platelets provide an important source of BDNF for regenerating peripheral sensory neurons at the site of nerve injury (Fujimura, Altar et al. 2002).

Platelet BDNF content can change rapidly, suggesting that they are a dynamic repository of BDNF in peripheral blood and are able to release BDNF under certain physiologic requirements (Lommatzsch, Niewerth et al. 2007). The ability of BDNF to cross the blood-brain barrier has been demonstrated (Pan, Banks et al. 1998), suggesting that serum BDNF levels may reflect levels in the brain. Despite the presumption of a short half-life in the plasma, the interest remains in the possible ability of BDNF to cross the blood-brain barrier in certain physiologic conditions (Pan, Banks et al. 1998; Schabitz, Steigleder et al. 2007). A recent study has demonstrated that in rats BDNF crosses the blood-brain barrier from brain to periphery and *vice versa*, since BDNF brain tissue and serum concentrations have been positively correlated (Sartorius, Hellweg et al. 2009). The authors even speculate that a main portion of elevated serum BDNF after electroconvulsive treatment has been derived from the brain with a time delay of three to seven days to establish an equilibrium again (Sartorius, Hellweg et al. 2009). Due to size and charge of BDNF it is possible that only a minimal amount might cross the blood-brain barrier via peripheral administration (Neto, Borges et al. 2011). On the other hand peripheral BDNF levels may only give an indirect hint on effective BDNF concentrations in the CNS. However, in principle a correlation of peripheral BDNF with cortical BDNF levels has been shown in studies with rats (Karege, Schwald et al. 2002). Furthermore, after peripheral administration in mice BDNF has been stable in circulating blood for 60 minutes and crossed the blood brain barrier via influx. It has been suggested that this rapid, saturable influx occurs through a specific transport system

(Pan, Banks et al. 1998). Until now, this finding has not been replicated and there is no study reporting a distinct possible transporter or transport mechanism for BDNF from blood to brain.

A.2.1 Transcription, trafficking, secretion and cleavage

The *Bdnf* gene is located on the short (p) arm of chromosome 11 at position 13 (11p13) and its genomic structure is quite complex. BDNF is the result of translation of at least 34 mRNA transcripts produced by alternative splicing of 11 upstream exons (exon 1-9a). Each of these exons code for the 5´untranslated region (5´UTR), linked to individual promoter regions. Splicing leads to a common downstream exon 9 (Figure 2) that encodes the BDNF pre-protein amino acid sequence and two

Figure 2: Illustration of the human *Bdnf* gene structure and its splicing variants. Exons are indicated by blue boxes. Dotted box in exon 9 indicates the coding region of the *Bdnf* gene. Arrows indicate alternative polyadenylation sites (Poly A) in the 3'UTR and internal alternative splice sites in exons 2, 6, 7 and 9a (letters a, b, c and d). The *Bdnf* gene is transcribed from different promoters, immediately preceding each of the 5' exons, that each full-length transcript contains a unique 5' exon and common 3' exon that contains the BDNF coding sequence (CDS). (adapted from Baj and Tongiorgi (Baj and Tongiorgi 2009)).

different 3´UTR sequences (Pruunsild, Kazantseva et al. 2007). The transcription of each exon is driven by separate promoters in turn controlled by an array of signalling mechanisms, for example calcium, cAMP response element-binding protein (CREB) and hormones (Lu, Pang et al. 2005; Molteni, Calabrese et al. 2009). Furthermore, it has been demonstrated that the account of specific BDNF splice variants is controlled by a variety of epigenetic mechanisms, including DNA methylation and posttranslational modifications of histones (Lubin, Roth et al. 2008; Roth, Lubin et al. 2009). The regulation of specific promoters causes the temporal and spatial expression of specific BDNF transcripts (Lauterborn, Rivera et al. 1996), some of which can undergo trafficking and targeting to dendrites (Chiaruttini, Sonego et al. 2008).

Initially synthesized as proform (pro-BDNF) it is either cleaved into the mature (m-BDNF) neurotrophin or transported to the plasma membrane and released in an unprocessed manner.

After synthesis in the endoplasmatic reticulum, pro-neurotrophins need to be folded correctly, sorted into the constitutive or regulated secretory pathway, and transported to the appropriate subcellular compartment (Lu, Pang et al. 2005). The pro-domain of BDNF binds to sortilin, a receptor that is mainly intracellular (Petersen, Nielsen et al. 1997; Nielsen, Madsen et al. 2001) and controls the mode

of secretion (Chen, Ieraci et al. 2005), in the Golgi to facilitate proper folding of the mature domain. Sortilin is co-localized with BDNF in secretory granules in neurons, and interacts with two sub-regions: box 2, containing the Val66 amino acid and box 3, both of which are in the pro-domain of BDNF (Lu, Pang et al. 2005). A motif in the mature domain of BDNF binds to the sorting receptor carboxypeptidase E (CPE). This interaction sorts BDNF into large dense core vesicles (LDCVs), a component of the regulated secretory pathway. In the absence of this motif, BDNF is sorted into the constitutive pathway. After binary decision of sorting, BDNF is transported to the appropriate site of release, either in dendrites or in axons. In some cases the pro-domain is not intracellularly cleaved by furin or protein convertases and therefore released as pro-BDNF by neurons. Extracellular proteases, such as metalloproteinases and plasmin can subsequently cleave the pro-region to yield mature BDNF (Schweigreiter 2006). Many non-neuronal cells, such as smooth muscle cells, fibroblasts and astrocytes, may not express molecular components of the regulated secretory pathway and, therefore, secrete neurotrophins only constitutively (Lu, Pang et al. 2005). Regulated secretion is prevalent in neurons. Neurotrophin-containing secretory granules are transported to dendrites and spines, and secreted postsynaptically. On the other hand, neurotrohpin-containing LDCVs undergo anterograde transport to axonal terminals. Either pro-neurotrophins are intracellularly cleaved, followed by secretion, or secreted and followed by extracellular cleavage, but the extent of intracellular and extracellular processing of pro-BDNF is not exactly clear. However, pro-BDNF is less efficiently processed by intracellular proteases compared to other neurotrophins and secretion of pro-BDNF with respect to m-BDNF seems to prevail (Mowla, Farhadi et al. 2001). Another possibility is the secretion without subsequent cleavage (Lu, Pang et al. 2005). In addition to interacting with $p75^{NTR}$, secreted pro-neurotrophins might be degraded extracellular.

A.3 Stress, BDNF and mood disorders

In the 1930s, the term stress was lent from engineering: a measure of internal forces acting within a deformable body, by Hans Selye. In his translation to biology, he defined stress as the result of an organism´s failed attempt to respond appropriately to a physical challenge (Selye 1998). Since then, this definition has been elaborated to include physiological threats (Schulkin, McEwen et al. 1994). Furthermore, the pioneering work of John Mason on psychological stress (Mason 1959) has permeated both modern psychology and neuroscience.

Nowadays, stress is used as a model to study alterations of brain structure and function because mood disorders are often caused or exacerbated by acute or chronic stressful life events (Gold and Chrousos 2002). Therefore, stress paradigms have long been used to model these diseases. Physical or psychological stress increases serum glucocorticoid concentrations. In rodents depression-like symptoms can be produced by chronic administration of glucocorticoids (Gourley, Wu et al. 2008). A prominent mechanism by which the brain reacts to acute and chronic stress is activation of the hypothalamic-pituitary-adrenal (HPA) axis (Figure 3)

Neurons in the paraventricular nucleus (PVN) of the hypothalamus secrete corticotropin-releasing factor (CRF), which stimulates the synthesis and release of adrenocorticotropin (ACTH) from the anterior pituitary gland. ACTH then stimulates the synthesis and release of glucocorticoids (cortisol in humans and corticosterone in rodents) from the adrenal cortex (Figure 3). Glucocorticoids exert profound effects on general metabolism and also dramatically affect behaviour via direct actions on numerous brain regions. Several brain pathways, including the hippocampus via an inhibitory influence on the hypothalamic CRF-containing neurons and the amygdala, control the activity of the HPA axis.

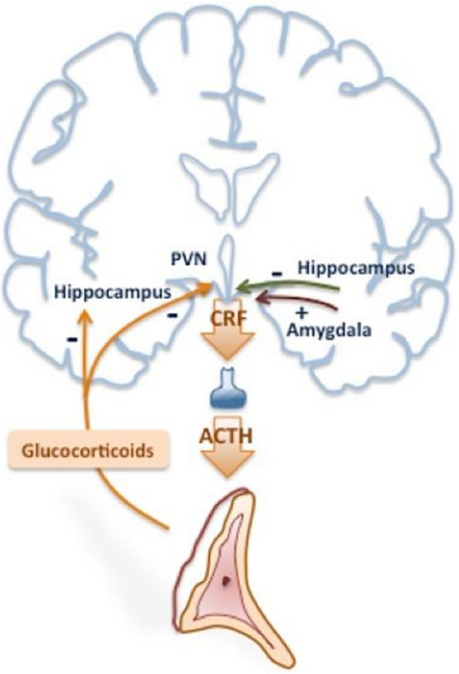

Figure 3: Regulation of the HPA axis. CRF-containing neurons of the hypothalamic PVN integrate information relevant to stress. The factor is released by these neurons into the hypophyseal portal system and acts on the anterior pituitary gland to release ACTH. This reaches the adrenal cortex via the bloodstream, where it stimulates the release of glucocorticoids (cortisol). In turn glucocorticoids repress CRF and ACTH synthesis and release and inhibit their own synthesis. At higher levels, glucocorticoids also impair, and may even damage, the hippocampus, which could initiate and maintain a hypercortisolemic state. **PVN** – paraventricular nucleus, **CRF** – corticotropin-releasing factor, **ACTH** – adrenocorticotropin (adapted from Nestler (Nestler, Barrot et al. 2002)).

Glucocorticoids, by potently regulating hippocampal and PVN neurons, exert powerful feedback effects on the HPA axis. Under normal physiological (McEwen 2000) conditions glucocorticoids seem to enhance hippocampal inhibition of HPA activity and even enhance hippocampal function in general, thereby promoting certain cognitive abilities (Nestler, Barrot et al. 2002). But, sustained elevations of glucocorticoids, seen under conditions of prolonged and severe stress, may damage hippocampal neurons (Nestler, Barrot et al. 2002), involve a reduction in dendritic branching and a loss of highly specialized dendritic spines where neurons receive their glutamatergic synaptic inputs (McEwen 2000; Sapolsky 2000).

Stress and the resulting hypercortisolemia can be manifested at several levels including i) impaired glucocorticoid-receptor-mediated negative feedback (Brown, Varghese et al. 2004), ii) adrenal hyper-

responsiveness to circulating ACTH (Parker, Schatzberg et al. 2003), iii) hyper secretion of CRF (Nemeroff and Owens 2002) and iv) the hypothalamic activator of ACTH release from the pituitary (de Kloet, Joels et al. 2005). As a result the inhibitory control that the hippocampus exerts on the HPA axis is reduced, which would further increase circulating glucocorticoid levels and subsequently damage the hippocampus in a positive feedback manner (Nestler, Barrot et al. 2002). Deregulation or hyperactivity of the HPA axis is one of the most prominent findings in up to 70% of patients with major depressive disorder (Porcelli 2011).

However, stress *per se* is not sufficient to cause a mood disorder like depression. Most people do not become depressed after serious stressful experiences, whereas some others do become depressed after stress that for most people is quite mild (Nestler, Barrot et al. 2002). This underscores, that stress associated mood disorders are caused by interactions between a genetic predisposition and environmental factors. A modulation of BDNF by stress was originally shown several years ago (Smith, Makino et al. 1995). Since then, evidence has been produced demonstrating the complex outcome of stress on the BDNF system and that the protein is a critical backbone in the functioning and well-being of the central nervous system.

Until now, several studies have demonstrated that stress causes impaired neurogenesis and atrophy in certain limbic structures, and that BDNF down-regulation is one of the events that occur. However, the precise mechanism underlying this down-regulation has not been fully understood. There is evidence that stressful experience decreases levels of specific BDNF isoforms, transcripts III and IV, associated with robust chromatin modification (Tsankova, Berton et al. 2006; Molteni, Calabrese et al. 2009) and therefore might contribute to atrophy of limbic structures, including the hippocampus, observed in depressive patients (Bremner, Narayan et al. 2000). Since the BDNF regulation system seems to be very complex because of several influencing factors, it has been suggested that glucocorticoids modulate BDNF signalling pathways. It is known that high adrenal-glucocorticoid levels, the hallmark endocrine response to stress, decrease BDNF expression. The hippocampus receives input from the HPA axis modulating stress responses and is important in emotional cognition and memory (McEwen 2005). BDNF and TrkB levels are decreased in regions of the hippocampus in *post-mortem* tissue taken from suicide victims, patients with major depressive disorder (MDD) or in the serum of MDD patients (Castren, Voikar et al. 2007; Castren and Rantamaki 2010; Thompson Ray, Weickert et al. 2011). Given that BDNF expression is decreased by stress, structural changes in the hippocampus related to MDD may be attributed in part to the reductions in BDNF and TrkB (Yu and Chen 2011).

Another region, the prefrontal cortex, essential to emotional processing, has been examined in relation to pathological features of MDD. In humans suffering from depression, this brain region has also been decreased in volume, correlated with lower BDNF and TrkB levels (Dwivedi, Rizavi et al. 2003; Castren 2004; Pandey, Ren et al. 2008). Paradoxical, BDNF expression is enhanced in other areas of the brain. Studies revealed that BDNF is increased in the nucleus accumbens (NAc) from human

patients with MDD (Krishnan, Han et al. 2007). Further findings suggest that BDNF is enhanced in the amygdala in response to stress (Yu and Chen 2011).

A.3.1 Major depressive disorder

Depression affects a huge number of people worldwide. Principal symptoms observed in depressed patients deal with loss of interest or pleasure, feelings of guilt or worthlessness, disturbed sleep or appetite, low energy, poor concentration and suicidal intentions (Fava and Kendler 2000; Nestler, Barrot et al. 2002). This unpleasant „state of mind" may be related with working conditions, self-perceived stress, anxiety and quality of life (Rusli, Edimansyah et al. 2008). However, it is curious why predisposition to develop a depression is higher in certain persons than in others. Today, it is assumed that a complex interaction between genetic, biochemical and environmental factors may be underlying the causative aetiology of this disorder (Nestler, Barrot et al. 2002). Epidemiologic studies show that roughly 40-50% of the risk for depression is genetic (Sanders 1999; Fava and Kendler 2000), including the fact that depression is a complex phenomenon with many genes possibly involved. In addition, vulnerability to depression is only partly genetic, with non-genetic and epigenetic factors also being important.

Several brain regions and circuits regulate emotion, reward and executive function. Dysfunctional changes within these highly interconnected limbic regions have been implicated in depression and antidepressant action (Berton and Nestler 2006). The monoamine hypothesis of depression was the most accepted by the scientific community (Hirschfeld 2000; Van Praag 2001; Owens 2004). This hypothesis has been based on the acceptance that the illness is caused by a deficit in the neurotransmission of serotonin and noradrenalin and that it could be reversed by drugs – namely antidepressants – that promote increase of these neurotransmitters in the synaptic cleft (Hyman 1993; Hindmarch 2002; Krishnan and Nestler 2008). However, this theory was not sufficient to explain the pathological mechanisms underlying depression. Antidepressants promote an immediate increase of serotonin and noradrenaline transmission acting either by blockage of the reuptake of monoamines, or by inhibition of their degradation at the synaptic cleft (Hindmarch 2002; Krishnan and Nestler 2008). Of note, the antidepressant effect is usually only observed after a few weeks of treatment adaptation, since several neurotransmitter systems next to downstream serotonergic or noradrenergic signalling pathways might be involved and are responsible for antidepressant efficacy. Interestingly, monoamine depletion studies demonstrate decreased mood in subjects with a family history of major depression (MD) and in drug-free patients with MD in remission, but do not decrease mood in healthy humans (Delgado, Price et al. 1991; Ruhe, Mason et al. 2007). Therefore, it becomes obvious that depression involves further modifications besides initial effects those at the monoamine system.

A.3.2 The neurotrophic hypothesis of depression

Over the past 10 years, molecular and cellular studies of stress, depression and antidepressants have moved the field of mood disorder research beyond the monoamine hypothesis of depression. These

studies demonstrate that stress and antidepressant treatment exert opposing actions on the expression of specific neurotrophic factors in limbic brain regions involved in the regulation of mood and cognition. Volumetric decreases observed in the hippocampus and other forebrain regions in subsets of depressed patients have supported a popular hypothesis for depression involving the decrease of neurotrophic factors (Monteggia, Barrot et al. 2004; Duman and Monteggia 2006). Many animal studies have also documented that stress reduces the expression of BDNF mRNA in the hippocampus (Smith, Makino et al. 1995; Duman and Monteggia 2006). Conversely, numerous classes of chemical antidepressants, as well as other forms of therapeutic interventions like electroconvulsive shock treatment and sleep deprivation, can significantly increase BDNF mRNA expression in hippocampus, prefrontal cortex or both of rodents (Nibuya, Morinobu et al. 1995; Russo-Neustadt, Beard et al. 2000; Duman and Monteggia 2006). This increase depends on chronic antidepressant treatment as shown in rats (Nibuya, Morinobu et al. 1995), which is consistent with the slow onset of therapeutic effects of antidepressants in a clinical setting. Furthermore, limited studies have shown that direct hippocampal infusion of BDNF protein can produce antidepressant effects in rodents (Siuciak, Lewis et al. 1997; Shirayama, Chen et al. 2002). These studies support the "neurotrophin hypothesis of depression", which postulates that reduced brain levels of BDNF could contribute to atrophy and cell loss in the hippocampus and prefrontal cortex, as observed in depressed subjects. Antidepressants may exert their therapeutic effects by increasing BDNF expression, thereby leading to the reversal of neuronal atrophy and cell loss (Duman and Monteggia 2006). The reduction of BDNF appears to be mediated partly via stress-induced glucocorticoids and partly via other mechanisms, such as stress-induced increases in serotonergic transmission (Smith, Makino et al. 1995; Vaidya, Marek et al. 1997). Compared to healthy human subjects, levels of BDNF are lower in *post-mortem* brain tissue from depressed patients but higher in those who were under antidepressant medication at the time of death (Chen, Dowlatshahi et al. 2001). Given that BDNF expression is decreased by stress and related to mood disorders, increased by antidepressants, and normalized in patients taking antidepressants, many investigations focused on BDNF as a "biomarker" and also a potential target for treatment of major depression. In sum, support for this BDNF hypothesis originates from large preclinical literature showing that several forms of stress reduce BDNF-mediated signalling in the hippocampus, whereas chronic treatment with antidepressants increases BDNF signalling (Nestler, Barrot et al. 2002; Duman and Monteggia 2006). Despite all these data, BDNF alone may not be sufficient to explain depression-related behaviours, but it remains an important risk factor.

A.3.3 BDNF and antidepressants / mode of action

The BDNF hypothesis predicts that agents that promote BDNF function might be clinically effective antidepressants. A causal role for the antidepressant action of BDNF has come from experiments in rodents in which antidepressant effects were observed on direct infusion of BDNF in the hippocampus (Shirayama, Chen et al. 2002) and were blocked on the conditional or inducible knockout of the gene encoding BDNF from forebrain regions (Monteggia, Barrot et al. 2004; Groves 2007). The time delay

for the therapeutic action of antidepressant treatment suggests that adaptations of receptor-coupled signal transduction proteins and their corresponding genes could contribute to the actions of antidepressants. BDNF modulation could be a key step in this adaptive process.

It has been shown (Siuciak, Boylan et al. 1996) that infusion of BDNF either intracerebroventricularly or directly into the rat midbrain produced analgesia and, interestingly, increased the activity of the monoaminergic systems. Indeed, BDNF infusion promotes the function, sprouting and growth of serotonin-containing neurons in the brain of adult rats (Altar 1999) and increases noradrenaline levels in several brain areas including the hippocampus (Siuciak, Boylan et al. 1996). These effects of BDNF on serotonergic and noradrenergic systems link the classical monoaminergic hypothesis of depression with the neurotrophic theory. Further studies demonstrated that both acute or sub-chronic (3-7 days) BDNF infusion into the hippocampus (DG and CA3 layer) or in the midbrain, produces an antidepressant-like effect in two behavioural models of depression, the learned helplessness and the forced swimming test paradigms. However, this effect seems to be specific to certain brain areas because BDNF infusion into the ventral tegmental area (VTA) or in the nucleus accumbens (NAc), for example, increases depression-like behaviour. Interestingly, this behaviour is reversed by the inhibition of BDNF signalling producing an antidepressant-like effect (Eisch, Bolanos et al. 2003). These findings are in line with the stress-induced increase of BDNF expression in prefrontal cortex (PFC) (Lee, Duman et al. 2006) and are opposite to the effect observed in the hippocampus, suggesting that BDNF antidepressant effect is area-dependent. Consistent through the literature is the finding, that BDNF infusion in the hippocampus or/and the midbrain produces antidepressant-like behavioural effects and that most of clinically effective antidepressant drugs work through this mechanism. *Post-mortem* tissue studies demonstrate that BDNF levels are increased in the hippocampus and cortex after long-term antidepressant use. In addition, studies of serum BDNF levels are normalized in patients suffering from depression after long-term antidepressant treatment (Duman and Monteggia 2006), a finding that has been validated after meta-analyses of multiple studies (Sen, Duman et al. 2008). However, studies of BDNF in *post-mortem* tissue are correlative, and the exact origin and function of serum-derived BDNF remains unclear. The *Bdnf* gene is induced *in vitro* and *in vivo* by CREB (Tao, Finkbeiner et al. 1998; Conti, Cryan et al. 2002). Moreover, virtually all major classes of antidepressants increase levels of CREB expression and function in several brain regions including hippocampus (Nibuya, Morinobu et al. 1995). Indeed, BDNF increase has been reported for selective serotonin reuptake inhibitors (SSRIs), noradrenaline reuptake inhibitors (SNRIs) (dual-action and tricyclic antidepressants), with monoamine oxidase inhibitors (MAOIs), atypical antidepressants, as well as with electroconvulsive shock treatment, one of the most clinically effective treatments for refractory depression (Nibuya, Morinobu et al. 1995; Kuroda and McEwen 1998; Russo-Neustadt, Beard et al. 1999; Fukumoto, Morinobu et al. 2001; Coppell, Pei et al. 2003; Holoubek, Noldner et al. 2004; Jacobsen and Mork 2004; Vinet, Carra et al. 2004; Song, Che et al. 2006; Garcia, Comim et al. 2008; Garcia, Comim et al. 2009; Larsen, Mikkelsen et al. 2010). In addition to improve or just

modify signalling pathways, antidepressants may help critical systems to overcome challenging conditions regarding neuronal plasticity, which is required to cope and adapt to stressful situations. It has been reported that antidepressants appear to increase the tyrosine autophosphorylation of TrkB receptors, activate PLCγ signalling and subsequent phosphorylation of CREB (Castren, Voikar et al. 2007). However, it is not clear how antidepressants modify BDNF levels. Probably the effects on BDNF are complex and may occur at several levels. It has been shown that changes of specific BDNF isoforms occur during antidepressant treatment (Molteni, Calabrese et al. 2009) that may contribute to an adequate response to stress. In conclusion, antidepressants may have a potential impact on activity-dependent plasticity within regions involved in emotional processing, affected in depression. Antidepressants may promote neuroprotective pathways and render them more responsive to preserve cell functionality. However, current results from the literature compelled a revision of the hypothesis regarding the antidepressant action of BDNF. First, a substantial number of preclinical studies either failed to show these patterns of changes induced by stress and by antidepressants, or have shown the opposite effects (Groves 2007; Martinowich, Manji et al. 2007). Second, male mice with conditional forebrain deletions of BDNF or its receptor do not show depression-like behaviour (Zorner, Wolfer et al. 2003; Monteggia, Luikart et al. 2007). Third, in other regions, for example the VTA and the NAc, BDNF exerts a potent pro-depressant effect: chronic stress increases the amount of BDNF within the NAc (Berton, McClung et al. 2006), and direct infusion of BDNF into the VTA-NAc increases depression-related behaviours (Eisch, Bolanos et al. 2003; Krishnan, Han et al. 2007). Finally, the single-nucleotide polymorphism Val66Met, which impairs intracellular trafficking and activity-dependent release of BDNF (Egan, Kojima et al. 2003; Chen, Jing et al. 2006) and decreases hippocampal volume (Szeszko, Lipsky et al. 2005; Chen, Jing et al. 2006) does not alter genetic vulnerability to depression (Gratacos, Gonzalez et al. 2007; Lopez-Leon, Janssens et al. 2008). Together these results suggest that the current formulation of the BDNF hypothesis is too simplistic. BDNF-mediated signalling is involved in neuroplasticity responses to stress and antidepressants, but these effects are both region-specific (Nestler and Carlezon 2006) and antidepressant-specific (Duman and Monteggia 2006). Of note, treatment with antidepressants, possibly through the actions of CREB or other transcriptional regulators (Nestler, Barrot et al. 2002; Pittenger and Duman 2008) increases the amounts of several growth factors, including BDNF (Sairanen, Lucas et al. 2005) in the hippocampus that influence neurogenesis. A marked cellular effect of several, but not all, antidepressant treatments is the induction of adult hippocampal neurogenesis (Sahay and Hen 2007; Pittenger and Duman 2008).

A.4 Pre- and clinical evidences: BDNF de-regulation in psychiatric disorders

Stress is a risk factor for major depression in vulnerable individuals. Basic research has used animal models, which imply stress to model such complex multi-syndrome psychiatric illnesses as partly aforementioned in paragraph A.3.2. Chronic stress is a generally accepted model of depression

because it leads to neurochemical and behavioural alterations that are analogous to those observed in depressed human patients, including increases in stress hormones, hippocampal atrophy, increased anxiety- and depression-related behaviours, and cognitive impairments (McEwen and Magarinos 1997; Yan, Cao et al. 2010). In general, most of the studies have shown that acute or chronic stress induced by different types of stressors, such as immobilization, unpredictable foot shock, social isolation, social defeat, maternal deprivation, restraint and swim stress decrease BDNF levels in the hippocampus (Choy, de Visser et al. 2008).

A.4.1 Animal models

A.4.1.1 Transgenic BDNF mice

Meanwhile multiple animal models of deficient BDNF signalling have been produced. Investigators generated heterozygous, conditional, and region-specific knockout or knockdown models to study depression-related behaviour in adult mice, since constitutive BDNF knockout mouse models show developmental brain abnormalities and die soon after birth (Ernfors, Lee et al. 1994). BDNF heterozygous mice reveal about 50% reduction of mRNA and protein throughout the animal. Furthermore, conditional and inducible genetic models have been developed to remove BDNF in a regionally and temporally dependent manner, to reduce profound changes in depression-related behaviour at later development (Chourbaji, Hellweg et al. 2004) and over the course of development for baseline-behaviour. In mouse models where BDNF is deleted from forebrain neurons later in development, there are no severe changes in depression-related behaviour (Monteggia, Barrot et al. 2004), although female mice may display behavioural alterations in certain assays (Monteggia, Luikart et al. 2007). This may be explained, because with this type of deletion behavioural effects cannot be attributed to specific neural circuits (Autry and Monteggia 2012). Though, all of these lines of mice consistently display an inability to respond to antidepressant treatment, pointing towards an essential role for BDNF in the presence of behavioural antidepressant responses (Monteggia, Barrot et al. 2004; Malberg and Blendy 2005; Tardito, Perez et al. 2006; Monteggia, Luikart et al. 2007). To target specific brain regions, viral-mediated deletion techniques are used to delete BDNF from regional restricted brain regions (Autry and Monteggia 2012). It has been shown that baseline depression behaviour in the dentate gyrus sub-region of the hippocampus has not been altered, even if dentate gyrus expression of BDNF is required for antidepressant efficacy (Adachi, Autry et al. 2009). Of note, specific deletion in the ventral tegmental area (VTA) exerts an opposing effect, resulting in an antidepressant-like response (Berton, McClung et al. 2006; Krishnan, Han et al. 2007). With the background of stress towards BDNF and the susceptibility to develop depression-related behaviours, it is accepted that BDNF plays an important role. However, how loss of BDNF alters vulnerability to stress has not been under investigation (Advani, Koek et al. 2009; Autry, Adachi et al. 2009). Some studies, report that BDNF heterozygous mice show altered depression-related behaviour after acute or sub-chronic stress (Advani, Koek et al. 2009). Conditional or inducible BDNF mutants suggest that

depression behaviour in male mice from these lines is indistinguishable from that in control mice after chronic mild stress (Ibarguen-Vargas, Surget et al. 2009).

A.4.1.2 Animal models related to stress

After chronic social stress, BDNF deletion in the VTA reduces depression-related behaviour (Berton, McClung et al. 2006). These inconsistencies suggest differences in circuitry or BDNF/pro-BDNF functions (Autry and Monteggia 2012). An overview of recent, though inconsistent studies assessing stress paradigms and hippocampal BDNF expression in rodents is shown in Table 1.

Similar to findings in human tissue, BDNF mRNA and protein expression are increased after long-term antidepressant therapies, such as electroconvulsive therapy, and many drugs, including SSRIs, norepinephrine reuptake inhibitors, tricyclic antidepressants, and atypical compounds in corticolimbic brain areas, including the hippocampus in animal models (Nibuya, Morinobu et al. 1995; Altar, Whitehead et al. 2003; Balu, Hoshaw et al. 2008). Additionally, infusion of BDNF into the midbrain, ventricles, or regions of the hippocampus results in increased antidepressant-like behaviour

Table 1. Stress paradigms to mimic depression related behaviour in animal studies. The effects on BDNF expression are indicated with arrows to mark an increase or decrease. Though, it is accepted that BDNF plays an important role to develop stress-related behaviour in mice and rats, data from the literature are inconsistent, suggesting differences in circuitry of BDNF functions. [1] (Smith, Makino et al. 1995), [2] (Nibuya, Morinobu et al. 1995), [3] (Ueyama, Kawai et al. 1997), [4] (Rasmusson, Shi et al. 2002), [5] (Barrientos, Sprunger et al. 2003), [6] (Pizarro, Lumley et al. 2004), [7] (Schulte-Herbruggen, Fuchs et al. 2009), [8] (Roceri, Hendriks et al. 2002), [9] (Roceri, Cirulli et al. 2004), [10] (Xu, Luo et al. 2004), [11] (Murakami, Imbe et al. 2005), [12] (Bergstrom, Jayatissa et al. 2008).

paradigm	duration	effect on BDNF	year	reference
stress				
immobilization	1, 7 days (45 minutes / day)	↓	1995	[1]
immobilization	45 minutes	↓	1995	[2]
immobilization	8 hours	↓	1997	[3]
footshock	60 minutes (0,4 mA)	↓	2002	[4]
social isolation	6 hours	↓	2003	[5]
social defeat	10 minutes	↓	2004	[6]
social defeat	5 weeks	↑	2009	[7]
maternal deprivation	24 hours, P9	↓	2002	[8]
swim stress	10 minutes / day, 14 days	↓	2004	[9]
restraint	4 hours / day, 3 days	↓	2004	[10]
restraint	6 hours / day, 21 days	↓	2005	[11]
restraint	1 hour / day, 7 days	↑	2008	[12]

(Shirayama, Chen et al. 2002; Hu and Russek 2008). These findings are supported by the overexpression of dominant-negative TrkB, which leads to loss of antidepressant efficacy, suggesting that TrkB activation is required for antidepressant behavioural effects (Saarelainen, Hendolin et al. 2003).

A.4.2 BDNF in depressed patients

To measure BDNF in human subjects, researchers use either blood or *post-mortem* brain samples. In *post-mortem* studies, reductions in the expression of pro-BDNF were also seen unilaterally in the hippocampus, but not in the DG, of subjects suffering from depression (Dunham, Deakin et al. 2009). The first investigations regarding the relation between BDNF and depression were cross-sectional studies like on the one hand simple assessment of current serum BDNF levels of treated and non-treated depressed patients and healthy controls (Karege, Perret et al. 2002; Shimizu, Hashimoto et al. 2003; Ziegenhorn, Schulte-Herbruggen et al. 2007). On the other hand, cohort studies assessed serum (Gervasoni, Aubry et al. 2005; Hellweg, Ziegenhorn et al. 2008; Okamoto, Yoshimura et al. 2008; Piccinni, Del Debbio et al. 2009) and plasma BDNF levels (Piccinni, Del Debbio et al. 2009) over time course before and after a defined antidepressant treatment. Further studies have been conducted as randomised controlled trial (RCT), the highest standard for clinical studies, to compare the influence of several antidepressant drugs on serum BDNF levels. (Ziegenhorn, Schulte-Herbruggen et al. 2007; Huang, Lee et al. 2008; Basterzi, Yazici et al. 2009; Gorgulu and Caliyurt 2009; Matrisciano, Bonaccorso et al. 2009). In the majority of clinical studies BDNF levels have been found lower in serum (Karege, Perret et al. 2002; Shimizu, Hashimoto et al. 2003; Huang, Lee et al. 2008; Sen, Duman et al. 2008; Gorgulu and Caliyurt 2009; Matrisciano, Bonaccorso et al. 2009) or plasma (Lee, Kim et al. 2007; Dreimuller, Schlicht et al. 2012) in depressed patients. In some cases these reductions are correlated with higher scores in specific depression evaluation scales (Gorgulu and Caliyurt 2009). Of note, not all studies show lower serum BDNF levels in depressed patients (Basterzi, Yazici et al. 2009). Nonetheless, there are some exceptions with a few studies reporting no differences in plasma or serum BDNF levels between depressed patients and healthy controls (Fernandes, Gama et al. 2009; Gustafsson, Lira et al. 2009). Few studies also suggest a gender specificity regarding BDNF levels during depression (Huang, Lee et al. 2008; Ozan, Okur et al. 2010). Both, healthy and depressed males showed higher serum BDNF levels than female subjects (Ozan, Okur et al. 2010). Therefore, it is important to correct for possible confounders *inter alia* gender, age, time of blood withdrawal, smoking status and alcohol intake (Bus, Molendijk et al. 2011). Data regarding effects of antidepressants on systemic blood BDNF levels have been inconsistent too, even when related to identical chemical substance classes, like selective serotonin reuptake inhibitors (SSRIs) (Hellweg, Ziegenhorn et al. 2008; Matrisciano, Bonaccorso et al. 2009). Evidence from the literature seems to promote that a successful antidepressant therapy intervention is accompanied with a normalization of BDNF blood levels. However, recent studies do not show a general increase of BDNF levels restricted to antidepressant drugs (Hellweg, Ziegenhorn et al. 2008; Basterzi, Yazici et al. 2009; Matrisciano, Bonaccorso et al. 2009). A summing up of literature about BDNF blood levels in depressed patients is listed in Table 2.

Table 2. Recent studies investigating blood BDNF levels with regard to depression and therapy intervention. Inclusion criteria are connected to diagnosis of depression and therapy interventions. Either serum or plasma has been assessed and changes of BDNF involving an increase, decrease or no change of BDNF levels are indicated with arrows. If antidepressants are not described in detail different substances of antidepressant classes were administered. [1] (Karege, Perret et al. 2002), [2] + [3] (Shimizu, Hashimoto et al. 2003), [4] (Gervasoni, Aubry et al. 2005), [5] (Aydemir, Deveci et al. 2005), [6] (Marano, Phatak et al. 2007), [7] (Yoshimura, Mitoma et al. 2007), [8] (Ziegenhorn, Schulte-Herbruggen et al. 2007), [9] + [10] (Hellweg, Ziegenhorn et al. 2008), [11] (Okamoto, Yoshimura et al. 2008), [12] (Gustafsson, Lira et al. 2009), [13] (Piccinni, Del Debbio et al. 2009), [14] (Basterzi, Yazici et al. 2009)], [15] (Matrisciano, Bonaccorso et al. 2009), [16] (Gorgulu and Caliyurt 2009), [17] (Diniz, Teixeira et al. 2010), [18] (Tadic, Wagner et al. 2011), [19] (Wolkowitz, Wolf et al. 2011), [20] (Birkenhager, Geldermans et al. 2012) , [21] (Gedge, Beaudoin et al. 2012), [22] (Dreimuller, Schlicht et al. 2012), [23] (Zhou, Xiong et al. 2013).

inclusion criteria	type	effect on BDNF	subgroups	year	reference
depression, antidepressants and BDNF					
depression	serum	↓		2002	[1]
depression	serum	↓		2003	[2]
depression + antidepressive therapy	serum	↑	treated vs. non-treated depressive subjects	2003	[3]
succesful antidepressive therapy	serum	↑		2005	[4]
depression + antidepressive therapy	serum	↑		2005	[5]
depression + electroconvulsive shock therapy	plasma	↑		2007	[6]
depression + antidepressant therapy	serum	↑	responder vs. non-responder	2007	[7]
depression	serum	↔		2007	[8]
depression + antidepressant therapy	serum	↓	SSRI	2008	[9]
depression + antidepressant therapy	serum	↑	tricyclic	2008	[10]
depression + electroconvulsive shock therapy	serum	↑	responder vs. non-responder	2008	[11]
depression + sport	plasma	↑		2009	[12]
depression + electroconvulsive shock therapy	plasma	↑	baseline vs. treatment time vs. controls	2008	[13]
depression + antidepressant therapy	serum	↔	SSRI vs. SNRI vs. controls	2009	[14]
depression + antidepressant therapy	serum	↑	SSRI vs. SNRI vs. controls	2009	[15]

depression + antidepressant therapy	serum	↑		2009	[16]
depression	serum	↓		2010	[17]
depression + antidepressant therapy	serum	↔		2011	[18]
depression + antidepressant therapy	serum	↑		2011	[19]
depression	serum	↔		2012	[20]
depression + electroconvulsive shock therapy	serum	↔		2012	[21]
depression + antidepressant therapy	plasma	↑		2012	[22]
depression	serum	↑	pro-BDNF vs. m-BDNF	2013	[23]

The underlying mechanism contributing to the augmentation of BDNF, upon antidepressant therapy are poorly studied in humans but might reflect changes in the brain. Nevertheless, one study (Cattaneo, Bocchio-Chiavetto et al. 2010) indicates that an increase of serum BDNF upon antidepressant treatment is associated with changes in BDNF mRNA levels in leukocytes, suggesting that these cells might play an active role in the mechanism of antidepressant action. Moreover, endurance training also induced an increase of BDNF levels in the hippocampus and enhanced release of BDNF from the human brain (Seifert, Brassard et al. 2010). This is in line with the thesis that physical exercise seems to have beneficial effects on depressed patients (Dimeo, Bauer et al. 2001; Pedersen, Pedersen et al. 2009; Conn 2010). Furthermore, sleep deprivation, as adjuvant to antidepressant therapy, seems equally to be associated with increases in BDNF levels (Gorgulu and Caliyurt 2009; Piccinni, Del Debbio et al. 2009).

A.4.3 BDNF polymorphisms in depression

The research for variations at the *Bdnf* gene has resulted in the identification of several single nucleotide polymorphisms (SNP) but the rs6265 has been the mostly studied until now. This functional polymorphism is located at nucleotide position 196 of the human *Bdnf* gene and is characterized by the substitution of a guanine to adenine base, which generates the replacement of valine by methionine at codon 66 (Val66Met) in the amino acid sequence. The Val66Met polymorphism has been associated with abnormal intracellular trafficking and regulated secretion of BDNF in cultured hippocampal neurons (Egan, Kojima et al. 2003). Furthermore, there is evidence that human subjects carrying the Met allele reveal abnormal hippocampal activation (Egan, Kojima et al. 2003), as well as poorer episodic memory (Egan, Kojima et al. 2003) and verbal recognition

memory (Goldberg, Iudicello et al. 2008). Other studies have equally shown smaller hippocampal volumes compared to Val/Val homozygotes (Szeszko, Lipsky et al. 2005; Bueller, Aftab et al. 2006; Montag, Weber et al. 2009). Furthermore, researchers identified SNP variations in the *Bdnf* and *NTRK2* (TrkB) genes that might be involved in antidepressant treatment outcome and that have been previously reported in this context (Hennings, Kohli et al. *in press*).

Whether the presence of specific BDNF polymorphisms may predispose the individual to develop major depression is still under investigation (Yulug, Ozan et al. 2010). Of note, animal studies support the idea that the Val66Met polymorphism may serve as a genetic predictor of future development of depressive disorders (Chen, Jing et al. 2006; Chen, Bath et al. 2008). In humans this is supported by some studies indicating that Met66 allele carriers are more liable to have geriatric depression than do Val66 homozygote individuals (Hwang, Tsai et al. 2006; Taylor, Zuchner et al. 2007; Lin, Hong et al. 2009). Of note, the literature reports that BDNF Val66Met polymorphism has been associated with major depression (Licinio, Dong et al. 2009), a risk for suicidal behaviour (Sarchiapone, Carli et al. 2008; Schenkel, Segal et al. 2010) as well as with rumination in healthy adults (Beevers, Wells et al. 2009), a behaviour characterized by the tendency to brood and repeatedly think about negative information, that is correlated with depression (Donaldson, Lam et al. 2007). Despite these findings, other studies did not find an obvious association of this BDNF variant with the disease itself (Chen, Lawlor et al. 2008; Liu, Xu et al. 2009; Verhagen, van der Meij et al. 2010), but there seems to be a relation of Val66Met polymorphism to the levels of BDNF. Both, patients and healthy controls carrying the Val66Met polymorphism have been exhibited lower BDNF serum levels than Val homozygote subjects, regardless of gender (Ozan, Okur et al. 2010). It has been suggested that genetic factors play a key role in both variation of response to treatment and incidence of adverse effects to medication (Schosser and Kasper 2009). It could be shown that rs7103411, Val66Met (rs6265) and rs7124442 BDNF polymorphisms are related with worse response to antidepressant treatment over 6 weeks in major depression (Domschke, Lawford et al. 2010), particularly in the melancholic depression (for rs7103411 and Val66Met) and anxious depression (for rs7124442) clinical subtypes.

In conclusion, there is some clinical evidence for an important effect of the genetic variability in BDNF on the risk to develop depressive behaviours and depression-associated mood disorders, such as suicide. Data indicate that the Val66Met polymorphism is associated with alterations in brain anatomy and memory performance, and that it might play a role on the individual response to antidepressant therapy.

A.4.4 Neurotrophins in brain disorders with cognitive impairment

Apart from their role in the pathophysiology of depression, neurotrophins seem to be implicated in other neuropsychiatric diseases as well, suggesting they might be a common pathogenic factor. Epidemiological and neurobiological evidences support a strong relationship between depression and dementia and several common pathophysiological mechanisms have been described, some of them involving neurotrophin signalling (Caraci, Copani et al. 2010). In the initial stage of Alzheimer`s

disease (AD), for example, cognitive impairment is often accompanied by mood instability and depressive symptoms (Assal and Cummings 2002) and the prevalence of AD is higher in persons with a history of major depression (Kessing and Andersen 2004). Several evidences lead to the hypothesis that BDNF deficiency might be one of the bridges between AD and major depression (Tsai 2003). Indeed, beta-amyloid (Abeta) protein deposits, which are observed in AD patients, seem to be associated with changes in BDNF content in serum and in cortical regions. Analysis of BDNF content in the serum of patients at two different stages of AD dementia revealed that BDNF values are increased in early stages of AD, while they decrease with the course of the disease, correlating with the severity of dementia (Laske, Stransky et al. 2006). Transgenic mouse models of AD suggest that the decreased BDNF expression is dependent on the aggregation state of Abeta as well as on large Abeta oligomers (Peng, Garzon et al. 2009). Additional to the information available on the link between BDNF and AD, there is also evidence for changes in neurotrophin content occurring in the course of other neurodegenerative pathologies such as Parkinson`s disease, schizophrenia and bipolar disorder (Murer, Yan et al. 2001; Kapczinski, Frey et al. 2008; Manfredsson, Okun et al. 2009). By employing a multi analyte proteomics profiling in plasma samples from schizophrenic patients, it has been found that BDNF might be a candidate biological marker for schizophrenia (Domenici, Wille et al. 2010). However, there are studies which show no changes in plasma BDNF in schizophrenic patients compared to healthy controls (Shimizu, Hashimoto et al. 2003; Lee and Kim 2009) or decreased serum levels at the onset of the first episode (Chen da, Wang et al. 2009) and during relapse (Chen da, Wang et al. 2009). In sum, available data from the literature indicate that neurotrophins might be a link between depression and other neuropsychiatric disorders. This is particularly true for BDNF in the case of AD, whose co-prevalence with major depression is high. It has been proposed that therapeutic use of BDNF itself or of drugs targeting its production may constitute a valid alternative to treat depressed patients with cognitive impairment or AD concomitant with depression (Tsai 2003).

A.5 Resilience and neuroadaptation

The brain may be considered as a primary mediator and target of stress resiliency and vulnerability processes because it adapts to changing environments and regulates the behavioural and physiological responses to a given stressor. It constantly sorts relevant from irrelevant external inputs and engages body systems to respond to these changes. The concept of emotional or psychological resilience has been a keystone of psychiatric thinking in responses to trauma for many years (Rutter 1985).

In the brain, corticosteroids play a key role in adaptive plasticity, as well as in the damage resulting from allostatic (physiological changes) overload (Sapolsky and Pulsinelli 1985; McLaughlin, Roozendaal et al. 2000). Modulators that work synergistically promote adaptation or damage, including the mineralcorticoid (MR) and glucocorticoid (GR) receptors, which bind the same hormone (cortisol in humans and corticosterone in rodents) in the brain, though with a difference in affinity.

Acting in concert with glucocorticoids, neurotrophins, such as BDNF, play an important role (Chen, Bath et al. 2008). Chronic stress can decrease BDNF expression in the brain (Smith, Makino et al. 1995; Smith and Cizza 1996), via a complex relationship (Isgor, Kabbaj et al. 2004). The *Bdnf* gene is negatively regulated by activated corticoid receptors (Schaaf, Hoetelmans et al. 1997). As a result of BDNF polymorphisms or changes in BDNF levels, alterations in BDNF signalling can be considered as risk factor in the development of neuropsychiatric diseases (Bath and Lee 2006; Soliman, Glatt et al. 2010). On the other hand, compromised BDNF signalling may result in a lack of stress effect. BDNF haploinsufficient mice show shrunken dendrites in the CA3 region of the hippocampus when compared to wild type mice. These mice do not show further shrinkage of hippocampal dendrites when chronically stressed in contrast to wild type mice, which do show stress-induced shrinkage (Magarinos, Li et al. 2011). While such results may be explained by a floor effect, another possibility is that BDNF is a limiting factor in the ability of the brain to show plasticity (Horch, Kruttgen et al. 1999; Horch and Katz 2002). Thus trophic factors such as BDNF are facilitators of plasticity, and the outcome may be negative (e.g. epilepsy) (Heinrich, Lahteinen et al. 2011) or positive (e.g. recovery from depression) (Castren 2005) depending on other factors operating at the time.

It is accepted that depression, and associated cognitive impairment, may be a result of an inability to return to normal functioning following a stressful or distressing psychological or physical situation, and therefore may be an example of a reduction in the capacity for plasticity and/or lack of resilience (Karatsoreos and McEwen 2011). In a sense, brain circuits become somehow locked and only exogenous interventions may succeed in promoting recovery and ameliorating the behavioural effects (Karatsoreos and McEwen 2011). The shrinkage of brain areas occurring in prolonged depression and a lack of plasticity could be a failure of resilience. Treatments that increase brain plasticity can effectively mobilize a brain that has become stuck, improving the behavioural symptoms by treating an underlying problem of plasticity. However, enhancing brain plasticity for someone who is depressed and in a negative environment may lead to adverse outcomes, such as suicide (Castren and Rantamaki 2010). Moreover, BDNF may be a facilitator of negative plasticity, such as epilepsy (Kokaia, Ernfors et al. 1995; Scharfman 1997; Heinrich, Lahteinen et al. 2011).

Researchers have examined the role that BDNF plays in susceptibility to developing stress-related mood disorders, but preclinical investigations have not yet demonstrated how loss of BDNF alters vulnerability to stress (Advani, Koek et al. 2009; Autry, Adachi et al. 2009). Differences in observations are likely to arise as a result of variations in the type of stressors, duration of stress, choice of behavioural assay or endpoint, mouse strain, and brain pathway examined.

In the context of stress-related mood disorders, resilience refers to the capacity of an individual to avoid negative social, psychological and biological consequences that would otherwise compromise their psychological or physical well-being. Resilience in humans is reported to represent an active, adaptive process and not simply the absence of pathological responses (Charney 2004; Feder, Nestler et al. 2009). A principal mediator of the impact of stress on brain and behaviour is activation of the

HPA axis, which results in widespread hormonal, neurochemical and physiological alterations (Herman and Cullinan 1997). Glucocorticoids, released from the adrenal cortex as a consequence of HPA axis activation, interact with steroid receptors expressed throughout brain that function primarily as transcription factors to regulate cellular function beyond the time scale to acute stress effects. In detail, glucocorticoid receptors and mineralocorticoid receptors, which respond to glucocorticoids, are expressed at high levels in hippocampus, amygdala, PFC and other limbic and midbrain structures, where they modulate the neural circuitry and neuroendocrine systems that underlie behavioural responses to stress. The effects of stress on the HPA axis depend on the developmental timing of the stress, as well as other critical factors such as stress magnitude, type and duration (Russo, Murrough et al. 2012). Many stress-induced changes are adaptive, but some seem to be damaging. Impairment of normal HPA function to regulate and terminate stress responses can result in many forms of deregulation, as illustrated by sustained elevations of glucocorticoid levels, which further affect neuroendocrine systems including immune responses, metabolism, and reproduction.

A.6 Sleep and BDNF

Sleep is an important component of human homeostasis (Han, Kim et al. 2012). It is generally accepted that sleep disruption is associated with abnormal brain function. A recent study revealed a biological link between synaptic plasticity in the cerebral cortex and sleep homeostasis (Huber, Esser et al. 2007). A key follow-up study provided evidence that the degree of BDNF expression during wakefulness is causally linked to the extent of slow wave activity (SWA), a sensitive marker for sleep pressure and sleep need, in the subsequent rest period (Faraguna, Vyazovskiy et al. 2008). Since the body´s stress system plays a critical role in adapting to a continuously changing and challenging environment, it is an important question whether these systems are affected by sleep loss. As sleep is dependent on several neurotransmitter systems, it is not surprising that sleep complaints have been reported in more than 80% of patients with depression or schizophrenia and that sleep abnormalities are common in patients with Alzheimer´s or Parkinson´s disease (Wulff, Gatti et al. 2010). Sleep problems are common features in many stress-related mental disorders; problems that may lead to impairment of physical and mental health because sleep loss is often followed by higher stress vulnerability (Morin, Rodrigue et al. 2003). Activation of the HPA axis by infusion of CRH has been demonstrated to produce sleep disruption in normal individuals (Steiger 2002). Equally, disruption of sleep control has wide-spread effects on all aspects of neural and neuroendocrine function (Wulff, Gatti et al. 2010). These effects include impaired cognition, impaired emotions, metabolic abnormalities, reduced immunity and elevated risks of cancer and coronary heart disease (Wulff, Gatti et al. 2010). In general, in the beginning of sleep the activity of HPA axis is suppressed continually. In the latter part of sleep, the HPA secretory activity increases so it is close to the maximum circadian rhythm immediately after waking up. The prominent activity of the HPA axis and sympathetic nervous system influences the overall amount of rapid eye movement (REM) sleep (Vgontzas, Bixler et al.

2001). Therefore, the circadian regulation of stress hormones with the rise of ACTH and cortisol in the morning is the crucial control factor to regulate the end of sleep (Weibel, Follenius et al. 1995). The fact that beginning and end of sleep involve HPA axis activity and the close temporal relationship between the axis and sleep provides a clue to estimate the effects of stress on sleep (Han, Kim et al. 2012). Additionally, the immune system is also influential in the relationship between stress and sleep via cytokines, which act as signalling molecules of the immune system such as interleukin-1 beta (IL-1β), tumour necrosis factor (TNF), and interferon. Many cells in the body produce pro- and inflammatory cytokines, they regulate each other and are in turn, regulated by glucocorticoids and catecholamines (Sapolsky, Romero et al. 2000). It has been demonstrated that cytokine activities are under neuroendocrine control (Jankovic 1994) through light/dark secretion of the hormone melatonin (Maestroni, Conti et al. 1988). Significant circadian or diurnal variations have been demonstrated in serum cytokine and cytokine receptor expression profiles including IL-2, IL-10, IL-1β, IL-6, TNF-α, IFN-γ, and IFN receptors (Young, Matthews et al. 1995; Lundkvist, Robertson et al. 1998; Takane, Ohdo et al. 2002). Likewise, BDNF was shown to be subjected to diurnal variation, in men and mice (Bova, Micheli et al. 1998; Schaaf, Duurland et al. 2000; Begliuomini, Lenzi et al. 2008; Piccinni, Marazziti et al. 2008; Choi, Bhang et al. 2011; Hamatake, Miyazaki et al. 2011). Furthermore, a possible physiological co-regulation between plasma BDNF and cortisol levels was suggested (Begliuomini, Lenzi et al. 2008).

Insomnia is a patient-reported problem, considered as sleep disorder as defined by the DSM-IV, regarding difficulties falling asleep or maintaining asleep, e.g. numerous awakenings, difficulty returning to sleep after awakenings, or awakening too early and struggle to return to sleep (Buysse 2013). It affects up to one third of the general population and epidemiological and clinical studies have shown that a high number of insomnia subjects also suffer from a concomitant mood disorder mainly depression or an anxiety disorder (Buysse, Reynolds et al. 1994; Schramm, Hohagen et al. 1995; Breslau, Roth et al. 1996; Ohayon 1997; Ohayon, Caulet et al. 1997). Suffering from insomnia is reliably distinguishable from good sleep by self-reported sleep symptoms, such as sleep latency (time to fall asleep) or wakefulness after sleep onset of longer than 30 minutes (Lichstein, Durrence et al. 2003). Insomnia disorders have been considered as primary and secondary (comorbid), depending on whether the sleep problem is triggered by another medical or mental disorder or medication/substance use (Buysse 2013). It has been shown that insomnia and psychiatric disorders interact in multiple ways, evidenced by the fact that only insomnia is infrequent (Ohayon and Roth 2003). Stress related insomnia is transient and persists for only few days. In the clinical setting a major problem is chronic insomnia, which is also called physiological insomnia (Han, Kim et al. 2012). Abnormalities in sleep timing and sleep architecture are recognized as common co-morbidities in numerous psychiatric disorders (Wulff, Gatti et al. 2010). These changes are usually described as difficulties in imitating and maintaining sleep during the night. Of note, persistent insomnia increases the risk of relapse into a new major depressive disorder episode (Pigeon, Hegel et al. 2008). Interestingly, some of the tricyclic (e.g.

amitriptyline, imipramine, clomipramine) and non-tricyclic antidepressants (e.g. mirtazapine, trazodone) have very pronounced sedative effects, and are commonly used as hypnotic agents in individuals without depression (Wulff, Gatti et al. 2010). Therefore, part of the efficacy of some antidepressants can be attributed to their direct action on sleep. Until know, the causal link between insomnia and psychopathology is not fully understood.

Moreover, sleep deprivation (SD) plays a distinct role in antidepressant therapy intervention. Nevertheless, the therapeutic effect is uncertain and only transient for one or a few days (Giedke and Schwarzler 2002). Standard treatment is total sleep deprivation (TSD) where the patient stays awake for one whole night and the following day. During these 40 hours of continuous wakefulness the patient abstains from any napping (Giedke and Schwarzler 2002).A variant of TSD is partial sleep deprivations (PSD) (Schilgen and Tolle 1980). Generally, PSD is chosen for treatment intervention since a total deprivation of sleep for one night is extremely exhausting for the patient and is combined with huge effort of personnel requirements. In PSD, patients got to bed as usual and are woken up at 1.30am and remain awake till the next evening (approximately 20h of continuous wakefulness). Another possibility is to deprive the patients of sleep in the early morning hours, staying awake until 1.30am, then sleeping until 7.00, because at this time the circadian course of several body functions changes direction (Schilgen and Tolle 1980).

Human studies investigating the association of BDNF with sleep disorders, especially insomnia, are missing and results from animal studies are inconsistent (Cirelli and Tononi 2000; Sei, Saitoh et al. 2000; Guzman-Marin, Ying et al. 2006). Until now, there was only one study measuring serum and plasma BDNF levels in sleep apnoea patients – representing a very specific subgroup of sleep disturbance –, which were found to be similar between patients and controls (Staats, Stoll et al. 2005).

B. Manuscripts

B.1 BDNF: an indicator for insomnia?

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In the last decade, brain-derived neurotrophic factor (BDNF) has become increasingly accepted as a central mediator of the effects of stress on neuronal plasticity and its implication for psychopathology. Neurotrophic functions of BDNF are implicated with neuronal survival, learning, memory, appetite and sleep.[1,2] The neurotrophin hypothesis is based on these features and proposes that stress-related mental disorders result from stress-induced decreases in BDNF expression.[3] Sleep problems are common features in many stress-related mental disorders, problems that may lead to impairment of physical and mental health because sleep loss is often followed by higher stress vulnerability.[4] Thus, insomnia is very common among depressed patients.[5] Of note, in accordance with the 'neurotrophin hypothesis of depression', BDNF seems to be involved in major depression and antidepressant action.[6,7] Although a majority of studies have concentrated on specifying the role of BDNF in depression, the relation between BDNF and insomnia has not been a focus of recent research. Here we investigated serum BDNF levels of adults with current symptoms of insomnia and non-sleep disturbed controls. The study was approved by the local ethics committee of the University of Basel. The sample pool consisted of 50 adults (mean±s.d. age=54.66±11.63 years), including patients with a previous diagnosis of restless legs syndrome (RLS) or periodic limb movement (PLM) but without other neurological symptoms, and age-matched controls. The eligibility criterion for sleep-disturbed participants was to suffer from at least sub-threshold insomnia (n=26, including 19 patients previously diagnosed with RLS/PLM and 7 controls); participants qualified as sleep-healthy subjects (n=24, including 7 patients with previous RLS diagnosis and 17 controls) by scoring below the cut-off (sum score of 8) for sub-threshold insomnia according to the *Insomnia Severity Index*, which is based on the DSM-IV diagnostic criteria for insomnia. Consistent with previous work,[8] there was a significant difference in serum BDNF levels between smokers and non-smokers ($t(47)=3.066$; $P=0.004$, ns: age, BMI and sex). Therefore, smoking was included as a covariate in subsequent analyses. Of note, participants suffering from current symptoms of insomnia (n=26) exhibited significantly decreased serum BDNF levels compared with sleep-healthy controls (n=24; $F(1)=5.017$; $P=0.03$; Figure 1a). In

addition, serum BDNF levels were significantly correlated with severity of insomnia in all participants (n=50; r_p=-0.409; P=0.004; Figure 1). There were no differences in serum BDNF levels between participants on medication (RLS medications included pramipexol and ropinirol, hypnotics, antidepressants, antipsychotics and others) and those without such drugs. Furthermore, serum BDNF levels did not differ between those with previous RLS/PLM diagnosis (n=26, including 19 patients with and 7 patients without insomnia symptoms) and those without such diagnoses (n=24, including 17 controls without and 7 with insomnia symptoms), which supports the view that serum BDNF levels are associated with sleep independently of diagnosis. We found subjective sleep impairment to be associated with lower serum BDNF levels, whereas reported good sleep was related to higher serum BDNF levels, as shown for those suffering from current insomnia compared with sleep-healthy subjects. To confirm the relevance of our preliminary findings, we investigated an additional independent control sample of adults (n=12, male non-smokers) that had recovered from occupational burnout and after 12 weeks of aerobic exercise training. To assess insomnia, we used the sleep-related items of the *Beck Depression Inventory*. Again, serum BDNF levels were significantly lower in those reporting symptoms of fatigue (n=6) compared with sleep-healthy subjects (n=6; t=2.2625; P=0.025; Figure 1b) and were significantly correlated (n=12, r_p=-0.639; P=0.025) with the symptoms of tiredness and fatigue known to reflect malfunction of sleep as the prime cause of impairments in daily life. The results from these two different samples are not strictly comparable, given the different methods of assessing disturbed sleep wake regulation as insomnia and day time fatigue, but despite or perhaps because of this, we believe that these findings support the hypothesis of an increased stress

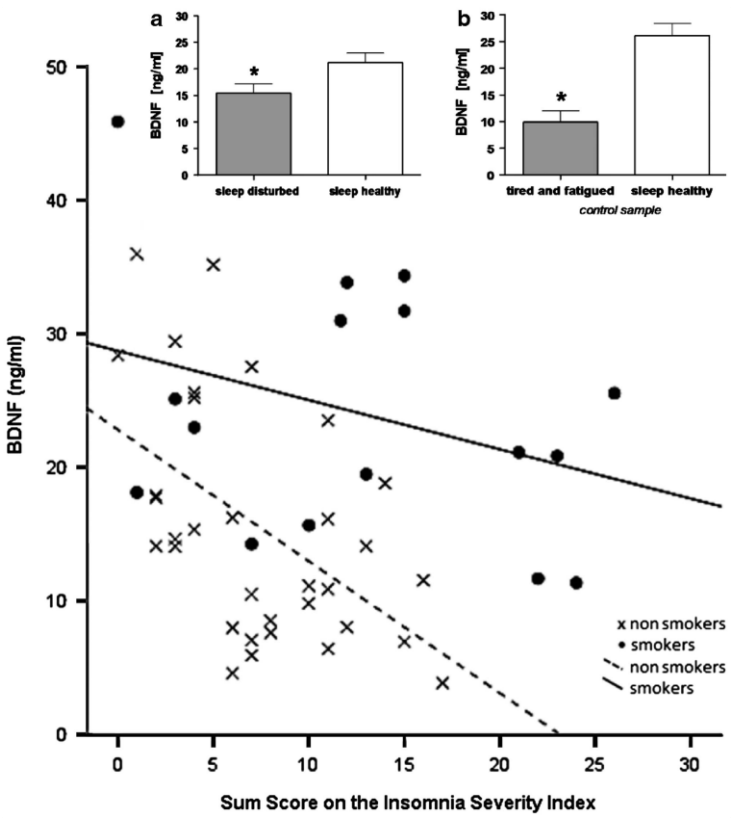

Figure 1. Correlation between serum brain-derived neurotrophic factor (BDNF) levels and insomnia severity index ratings. Analysis showed a significant correlation of BDNF levels with severity of insomnia across the sample as a whole (r_p=-0.409; P=0.004). Black circles represent smokers (r=-0.331; P=0.21) and open circles represent non-smokers (r=-0.511; P=0.002). (a) Plotted estimated means by ANOVA ± s.e.m. of serum BDNF levels controlled for smoking of sleep-disturbed (n=26; 15.41±1.77 ng ml^{-1}) and sleep-helathy (n=24; 21.15±1.80 ng ml^{-1}) subjects. (b) Means ± s.e.m. of serum BDNF levels of tired and fatigued (n=6; 9.86±2.16 ng ml^{-1}) and sleep-healthy (n=6; 26.08±2.30 ng ml^{-1}) subjects. * Denotes statistical significance at P<0.05.

vulnerability due to sleep loss, which may lead to decreased BDNF secretion. Such a decrease might be associated with a decrease in BDNF mRNA expression in peripheral blood mononuclear cells and/or may correspond to a decline of BDNF concentration in the brain. To what extent peripheral BDNF levels correspond to brain BDNF levels remains unknown. However, the use of serum BDNF concentration as potential indicator of brain alteration is justified by extensive evidence.[9] Although we report a reduction of BDNF levels linked to sleep disturbance, others have consistently shown that prolonged wakefulness as a result of sleep deprivation, which can be considered as a stressor for the brain, leads to an increase in BDNF.[10] Using a bidirectional stress model as an explanatory approach, we hypothesise that chronic stress induces a deregulation of the HPA system, leading in the long term to sleep disturbance and decreased BDNF levels, whereas acute sleep deprivation, for example, one night of sleep deprivation, can be used as therapeutic intervention in some insomniac or depressed patients as a compensatory process to normalize BDNF levels. BDNF has also been considered as a predictor of therapeutic response in major depression. However, very recent data indicate that the increase in serum levels of BDNF during antidepressant treatment appears to be confined to some but not all antidepressants and does not coincide with amelioration of clinical symptoms.[6] On the basis of our results, this discrepancy might stem from changes in central BDNF concentrations, following reduced insomnia rather than depressive symptoms and antidepressants differentially influencing sleep. Thus, our preliminary findings suggest that serum BDNF levels are not associated with a specific (categorical) diagnosis, but may be associated with insomnia symptoms independent of diagnosis (dimensional). In line with this, we suggest that, when analysing serum BDNF levels in depressed patients, insomnia symptoms should be carefully controlled, as well as improvements in sleep during therapy. Further studies with a larger number of patients and a design including objective measurements of sleep, such as sleep polysomnography, should be conducted to verify the results of our exploratory investigation and to elucidate the underlying mechanisms more closely, especially the role of the stress hormone system.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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B.2 The interplay of stress and sleep impacts BDNF level

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Short running title:

BDNF, Sleep and Stress

Manuscript submitted

ABSTRACT

Background: Sleep plays a pivotal role in normal biological functions. Sleep loss results in higher stress vulnerability and is often found in mental disorders. There is evidence that brain-derived neurotrophic factor (BDNF) could be a central player in this relationship. Recently, we could demonstrate that subjects suffering from current symptoms of insomnia exhibited significantly decreased serum BDNF levels compared with sleep-healthy controls.

Objective: In accordance with the paradigm indicating a link between sleep and BDNF, we wanted to investigate if the stress system influences the association between sleep and BDNF.

Method: Participants with current symptoms of insomnia plus a former diagnosis of Restless Legs Syndrome (RLS) and/or Periodic Limp Movement (PLM) and sleep healthy controls were included in the study. Participants completed questionnaires on sleep (ISI, *Insomnia Severity Index*) and stress (PSS, *Perceived Stress Scale)* and provided a blood sample for determination of serum BDNF levels.

Results: We found a significant interaction between stress and insomnia with an impact on serum BDNF levels. Moreover, insomnia severity groups and score on the PSS each revealed a significant main effect on serum BDNF levels. Insomnia severity was associated with increased stress experience affecting serum BDNF levels. Of note, the association between stress and BDNF was only observed in subjects without insomnia. Using a mediation model, sleep was revealed as a mediator of the association between stress experience and serum BDNF levels.

Conclusions: This is the first study to show that the interplay between stress and sleep impacts BDNF levels suggesting an important role of this relationship in the pathology of stress-associated mental disorders. It is already known that stress is a major effector of BDNF regulation. Hence, we suggest sleep as key mediator at the connection between stress and BDNF. If sleep is maintained or disturbed

might explain why some individuals are able to handle a certain stress load while others develop a mental disorder.

INTRODUCTION

Sleep is associated with physical and mental health. [1,2,3,4]. Sleep loss impairs various endocrine, physiological [5] as well as neuronal functions [6,7,8] and is often followed by higher stress vulnerability, reduced environmental adaptation and cognitive impairment [9]. Moreover, insomnia is often observed in many stress-related disorders [3]. Evidence indicates that BDNF could play a role in this association: i) in animal studies BDNF levels decreased after chronic stress [10,11,12,13], ii) serum BDNF levels decreased in stress-related major depressive disorder [14,15,16], iii) BDNF plays a role in sleep homeostasis [17,18,19]. Additionally, a recent study by our group showed that insomnia is associated with decreased serum BDNF levels [20].

The major stress response system, the hypothalamic–pituitary–adrenal (HPA) axis, facilitates the adaptation to stress. Chronic stress can lead to a deregulation in this biological stress system [21,22,23], which was suggested to influence BDNF levels in limbic brain structures in animal studies and in blood from the human periphery, namely serum and plasma [10,11,12,14,15,16]. In rodents it was shown that acute and chronic stress decreased levels of BDNF in the dentate gyrus and the hippocampus. This reduction seemed to be mediated partly via stress-induced glucocorticoids [24].

Next to the nervous system, BDNF is found in the periphery of humans and other mammals [25,26]. Since the protein can cross the blood brain-barrier in both directions, circulating BDNF correlates with cortical BDNF concentrations [27]. In addition to the classic functions of BDNF as neurotrophin, several studies involving human subjects and animal models provide preliminary data supporting a role for BDNF in stress and mood disorders [14,28,29,30,31].

Altogether, these findings suggest a possible role of the interplay between stress, sleep and BDNF however the relation remains unclear. Therefore the aim of this study was to test how stress and sleep could affect serum BDNF levels. To elicit our hypothesis, participants with current symptoms of insomnia and non-sleep disturbed controls were asked to complete questionnaires on insomnia and stress experience. In the same sample, we could already demonstrate an association between decreased serum BDNF levels and insomnia severity [20].

MATERIALS AND METHOD

Participants and Procedure

Participants were recruited in three different ways. First, patients with a former diagnosis of Restless Legs Syndrome (RLS) and or Periodic Limb Movement (PLM) were recruited from the Department of Sleep and Depression Research of the Psychiatric University Clinic Basel, Switzerland. Second, we

recruited participants using a *study participant database* provided by the Institute of Psychology of the University of Basel and third – in order to reach older participants – we asked members of an organization of the elderly to participate.

At first contact, the study was explained by phone, e-mail or face-to-face. When interested in participation, patients and controls were thoroughly informed about the study and received an information-package containing detailed study information and several questionnaires, which they were asked to complete at home. Participants were then invited to a personal appointment in the facilities of the Department of Sleep and Depression Research of the Psychiatric University Clinic Basel in Switzerland, where they were requested to arrive fasting in the morning at 7.45 a.m. to provide a blood sample and complete several questionnaires. The local ethics committee of Basel approved the study protocol. All participants gave written informed consent in accordance with the declaration of Helsinki and received financial compensation. A total of 50 participants were included in the study (for further details see Table 1 – characteristics of study participants). Besides a sociodemographic questionnaire to assess sex, age and BMI, participants also had to indicate substance consumption (coffee, cigarettes, alcohol, marihuana and other illegal drugs) and pharmaceutical intake, which could interfere with the biological analyses [32,33,34,35]. Marihuana and illegal drugs were ignored in further analyses, as no participant indicated to consumption any of these two. Only smoking was associated with serum BDNF levels and therefore included in subsequent analyses.

Table 1. Descriptive characteristics of study participants. A total sample size of N=50 were included in the analyses. Descriptive data are presented in means (*M*) and standard deviations (*SD*). Absolute numbers of participants are given (*N*) and expressed as percentage (*%*).

		M	*SD*
Age (years)		54.7	11.6
Body mass index		27.2	5.1
Serum BDNF (ng/ml)		18.01	9.84
Score on the ISI		9.4	6.6
Score on the PSS		26.5	7.4
		N	**%**
Sex	male	25	50
	female	25	50
Former diagnosis	none	24	48
	RLS/PLM	26	52
Cigarette smoking	smoker	16	32
	non-smoker	33	66
	missing	1	2

Abbreviations: means (M), standard deviation (SD), brain-derived neurotrophic factor (BDNF), Insomnia Severity Index (ISI), Perceived Stress Scale (PSS), restless legs syndrome (RLS), periodic limb movement (PLM)

BDNF analysis

For serum sampling, blood was obtained in a serum separator tube from the antecubital vein between 7.45 and 8.00a.m. After 30 minutes of clotting time, the whole blood was centrifuged at 1000xg for 30min to separate and collect the serum. Aliquots were kept at -80°C until assaying.

Serum BDNF levels (concentration: ng/ml) were assessed with an enzyme-linked immunoabsorbant assay (ELISA) kit (Promega BDNF Emax®, Madison, Wis.). Samples were appropriately diluted (between 1:100-1:150) and detection of total soluble BDNF was carried out in an antibody sandwich format like described in the manufacturers protocol. The absorbance was measured within 30 minutes in a microplate reader at 450nm to determine BDNF concentrations according to the standard curve. All assays were carried out in duplicates and means were calculated.

Questionnaires

SLEEP Participants completed the *Insomnia Severity Index (ISI)* [36], which is an established screening questionnaire for insomnia. The ISI is a 7-item scale that yields a quantitative index of insomnia severity and is partly based on the DSM-IV diagnostic criteria for insomnia [19]. Participants were asked to specify on a 5-point Likert scale ranging from 0 (= not at all) to 4 (= very much) to what extend they suffered from difficulties in falling asleep or maintaining sleep and early awakenings during the last two weeks. Additionally they are asked to rate satisfaction with sleep, daytime sleepiness and worrying about (bad) sleep. A sum score of 8 to 14 indicates that the respondent suffers from sub-threshold insomnia. A score of 15 and higher indicates clinical insomnia.
STRESS Stress perception was assessed using the German versions of the *Perceived Stress Scale (PSS)* [19], which consists of 10-items and is used to determine perceived overall stress occurring in the previous month. The German version of the PSS has satisfactory internal consistency and test-retest reliability [14]. Answers were given on a 5-point Likert scale ranging from 1 (= never) to 5 (= very often), with higher scores reflecting greater perceived stress.

Statistical Analysis

For descriptive purposes means and standard deviations and errors respectively were calculated for stress, sleep and serum BDNF levels and potential confounding variables.
First, potential confounders were examined and later included in the analyses if they were significantly correlated with scores on the ISI, PSS or serum BDNF levels (Pearson's (r) or Spearman's (rs) correlation coefficients). Second, we performed an ANCOVA to assess the main effects of sleep and stress and their interaction with regard to serum BDNF levels. Third, we conducted mediator models (see figure 3) using the bootstrap calculation of an SPSS macro according to Preacher and Hayes [37]. All analyses were performed using SPSS Statistics 20 for Macintosh. A *p*-value smaller than 0.05 was considered as significant.

RESULTS

Participant characteristics and potential confounders

Descriptive characteristics of participants are shown in Table 1. There was a significant association of BDNF with smoking (r_p = 0.408, p = 0.004) which was included as a covariate in further analyses. To assess the influence of insomnia severity we divided all participants into three subgroups according to their score on the *Insomnia Severity Index*: subjects with no insomnia (score 0-7, n = 24), sub-threshold insomnia (score 8-14, n = 16) and clinical insomnia (score 15-30, n = 10). Serum BDNF levels did not differ between diagnosis groups (RLS, PLM, sleep-healthy) and were not influenced by medication [20].

Serum BDNF levels, sleep and stress

Previously, we could show that an increase in severity of insomnia was associated with a decrease in serum BDNF levels of the same sample [20]. Serum BDNF levels in the group with no insomnia were significantly higher compared to the groups reporting sub-threshold and clinical insomnia (Figure 1a). As expected, stress experience on the PSS increased with rising sum score of insomnia severity (r_p=0.548; p<0.001) (Figure 1b).

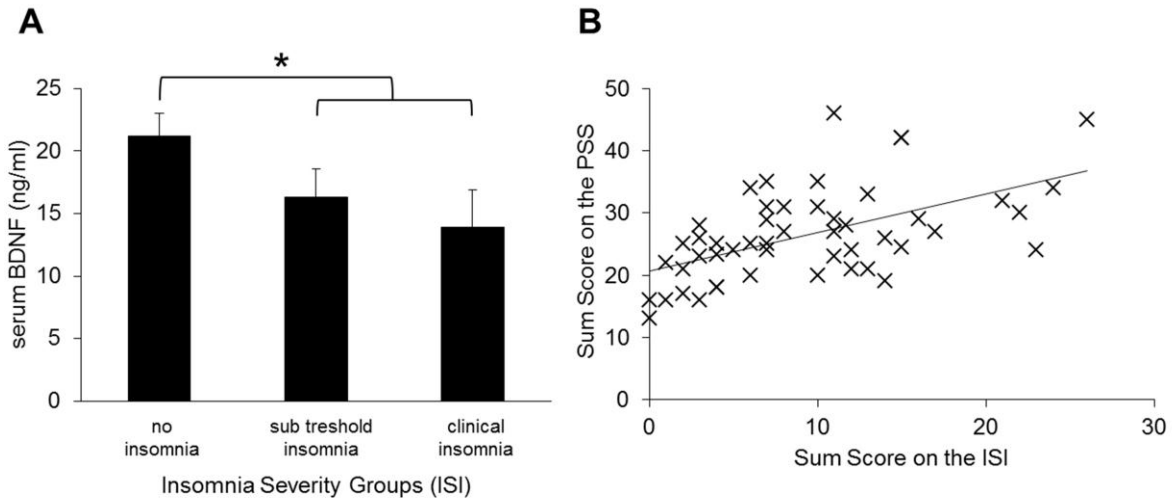

Figure 1. Serum BDNF levels and stress experience in subjects suffering from current insomnia. (A) Mean serum BDNF levels of the insomnia severity groups. Plotted means and standard errors estimated by ANCOVA with serum BDNF as dependent variable, insomnia severity group as independent variable and smoking as covariate. For all three insomnia severity groups the overall effect on serum BDNF was not significant (F(2)=2.67; p=0.080). Contrasts showed that serum BDNF levels in the group with no insomnia were significantly higher compared to the groups reporting sub-threshold and clinical insomnia (F(1)=5.33; p= 0.026); (no insomnia n=24; sub-threshold insomnia n=16, clinical insomnia n=10). (B) Correlation between insomnia severity score, indicated by *the Insomnia Severity Index* (*ISI*), and stress perception, indicated by the *Perceived Stress Scale (PSS)*. Analysis showed a significant correlation between scores on the ISI and the PSS across the whole sample (r=0.548; p<0.001). * Denotes statistical significance at p<0.05

To obtain a better understanding of the complex interplay between stress, insomnia and BDNF we calculated an ANCOVA (BDNF as dependent variable, insomnia and stress as independent variables and smoking as covariable). We found a significant interaction between stress and insomnia with an

impact on serum BDNF levels (F=6.180, p=0.017). Moreover we found a significant main effect for the independent variables: (i) insomnia severity groups (F=7.775, p=0.008) with an incremental decrease in serum BDNF levels from the no insomnia to the sub-threshold and to the clinical insomnia group, (ii) score on the PSS (F=8.230, p=0.006), with decreased serum BDNF levels associated with increased scores on the PSS and (iii) the covariate smoking (F=14.154, p<0.001). Of note, an association between the PSS and BDNF was only observed in subjects with no insomnia (r_p=-0.511, p=0.013) compared to subjects with sub-threshold (r_p=0.069, p=0.814) or clinical insomnia (r_p=0.199, p=0.608) (Figure 2).

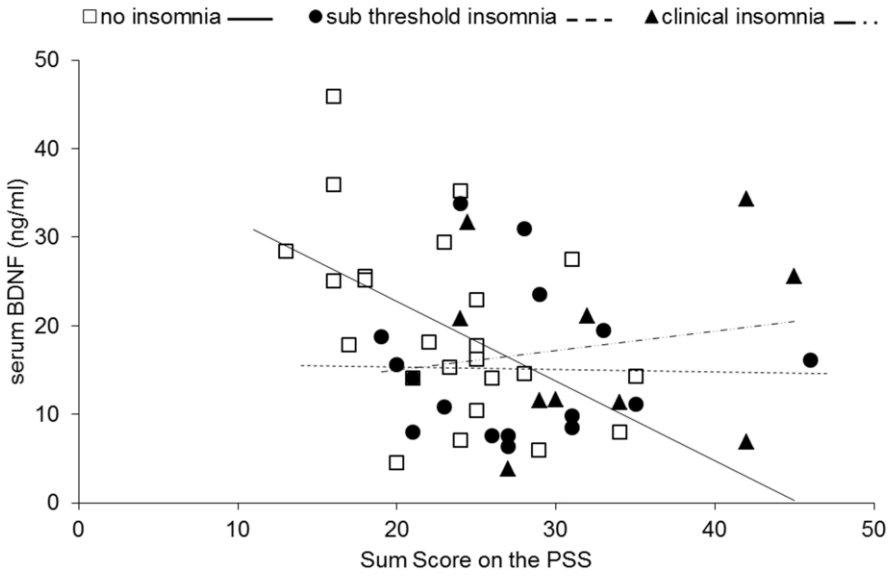

Figure 2. Correlation between serum BDNF levels and scores on the *Perceived Stress Scale (PSS)* by insomnia severity group according to the *Insomnia Severity Index (ISI)*. Analyses showed a significant correlation (partial correlation controlled for smoking) between BDNF and stress only in subjects with no insomnia (r_p=-0.511, p=0.013) compared to subjects with sub-threshold (r_p=0.069, p=0.814) or clinical insomnia (r_p=0.199, p=0.608). White squares represent subjects with no insomnia, black circles represent subjects with sub-threshold and black triangles represent subjects with clinical insomnia.

To further elucidate a mutual relationship between the three parameters stress, sleep and BDNF we calculated a mediation model as described by Preacher & Hayes [37]. In the first model (Figure 3a) we defined stress as mediator of the relationship between sleep and BDNF. Results showed a significant 'a' path (t=4.36; p<0.001) between sleep and stress. The 'b' path between stress and BDNF was not significant (t=-0.92); p=0.365). The 'c' path between sleep and BDNF was significant after inclusion of stress as mediator (t=-2.05; p= 0.046). The indirect 'ab' path was not significant (bootstrap 95% confidence intervals: lower =-0.385, upper =0.169, p>0.05). In the second model (Figure 3b) we defined sleep as mediator of the relationship between stress and BDNF. As expected, the 'a' path (t=4.36; p<0.001) between stress and sleep was significant. Here, also the 'b' path between sleep and BDNF was significant (t=-2.05; p=0.046). The 'c' path between stress and BDNF was not significant when the mediator sleep was included (t=-0.92; p=0.365) and this time the indirect 'ab' path was significant (bootstrap 95% confidence intervals: lower =-0.522, upper =-0.046; p<0.05). Both models

can explain 27.3% of the variation (adjusted R square) in the serum BDNF levels ($F(7, 45)=7.013$; $p<0.001$).

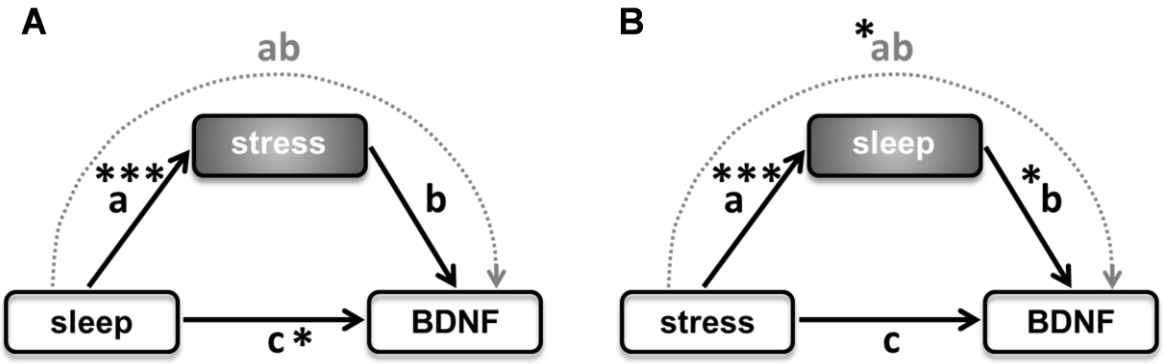

Figure 3. Mediation models for the interplay between stress, sleep and BDNF. (A) Stress as a mediator in the relationship between sleep and BDNF. Analyses revealed a significant 'a' path (t=4.36; p<0.001) between sleep and stress. The 'b' path between stress and BDNF was not significant (t=-0.92; p=0.365). The 'c' path between sleep and BDNF was significant after inclusion of stress as mediator (t=-2.05; p=0.046). The indirect 'ab' path was not significant (bootstrap 95% confidence intervals: lower =-0.385, upper =0.169, p>0.05). (B) Sleep as mediator in the relationship between stress and BDNF. The 'a' path between stress and sleep was significant (t=4.36; p<0.001), as was the 'b' path between sleep and BDNF (t=-2.05; p=0.046). The 'c' path between stress and BDNF was not significant when the mediator sleep was included (t=-0.92; p= 0.365). In this model the indirect 'ab' path was significant (bootstrap 95% confidence intervals: lower =-0.522, upper =-0.046; p<0.05). Both models explain 27.3% of the variation (adjusted R square) in serum BDNF levels (F(7.45)=7.013; p<0.001). Smoking was included as covariate in both models. * Denotes statistical significance at p<0.05 and *** p<0.001

DISCUSSION

The aim of this study was to investigate if the influence of the association between stress and sleep has an impact on serum BDNF levels. As previously shown, insomnia was correlated with decreased serum BDNF levels [20]. In the present study we found an interaction between stress and insomnia which affects serum BDNF levels. Interestingly, an association between stress and BDNF was only found in subjects without insomnia. Therefore the present results are in line with the assumption that stress somehow affects the link between sleep and BDNF.

To further elucidate this finding we investigated two mediator models (Figure 3a and b). In the first model, stress was defined as mediator of the relationship between sleep and BDNF and in the second model sleep was determined as the mediator of the relationship between stress and BDNF. The first model did not identify stress as a mediator of the relationship between sleep and BDNF. Notably, our second model revealed that sleep mediated the association between stress and BDNF. Importantly, this means that increased stress negatively affects sleep and in turn decreases BDNF levels. Our main finding is partly in line with previous research showing a major role for stress on BDNF regulation. Our results suggest for the first time sleep as a key mediator in the connection between stress and BDNF.

The results of our study revealed that only subjects who suffer from increased stress and at the same time from comorbid sleep disturbances show decreased BDNF levels. We argue that stressed while not

sleep-disturbed subjects have BDNF levels similar to non-stressed subjects. This highlights the importance of good sleep in dealing with stress. We assume that the interplay between decreased BDNF, chronic sleep impairment and increased stress levels is an essential mechanism in the pathology of stress-associated mental disorders. If sleep is maintained or disturbed might explain why some individuals are able to handle a certain stress load while others develop a mental disorder. Thereby, adequate levels of BDNF could promote neuronal plasticity, a factor supporting mental health.

Our study is the first to combine two predictors, sleep and stress, regarding BDNF levels. Moreover, we were able to bring sleep and stress in relation to each other. This is important since stress-related mood disorders are characterized by multifactorial features and are often comorbid with sleep disorders. Another strength is that we did not simply compare patients and controls by diagnosis but investigated insomnia severity in all subjects. Therefore we are able to make statements about the consequences of insomnia in general rather than related to a specific clinical diagnosis. This is important with regard to underlying mechanisms independent of a diagnosis. In sleep healthy subjects we saw a strong association between stress and serum BDNF levels. This could indicate that in these subjects stress results in decreased BDNF levels, while this association was diminished in sleep-disturbed subjects. A reasonable argument could be that chronic sleep loss, probably caused by stress, is associated with reduced circulating serum BDNF in general, as well as an impaired stress adaptation system.

This study has also some limitations. First, there could be various other biological factors influencing BDNF levels, which were not analysed in this study such as physical activity [38] alterations in neurotransmitter and hormone production and function, cytokines, [39]as well as genetic and epigenetic mechanisms [40,41]. Second, further studies should examine other biological correlates of sleep impairment, difficulties in stress coping and elevated stress perception. Third, studies on patients with specific sleep-related disorders or with experimentally induced sleep restriction have to be conducted to generalize these results. Therefore, we suggest that future studies should investigate – next to subjective psychological stress ratings – objective endocrine stress parameters, such as cortisol and a greater sample sizes should be aspired. Fourth, there might be additional cofactors that were not assessed, such as comorbidly existing somatic and psychiatric disorders or nutritional intake.

CONCLUSIONS

This is the first study to show that stress experience and subjective sleep perception interact with each other and affect BDNF levels. We suggest that this interplay is involved in the pathology of stress-associated mental disorders. We add new weight to our previous suggestion [20] to seriously consider the assessment of sleep when analysing BDNF as a marker in stress-related mood disorders, since several studies have found decreased levels of serum BDNF in various stress-related mental disorders, such as depression [14,42], posttraumatic stress disorder [43] and burnout syndrome [44], all of which

are associated with sleep-related problems. The underlying biological mechanisms e.g. involvement of the stress hormone system, have to be elucidated in future studies.

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B.3 A diurnal profile of serum BDNF before treatment is associated with therapy response after partial sleep deprivation in major depression

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Key words: **BDNF, sleep deprivation, depression, therapy response**

Manuscript submitted

ABSTRACT

Background: Brain-derived neurotrophic factor (BDNF) is an important member of the neurotrophin family of growth factors, abundant in the brain and periphery. Researchers have reported that serum BDNF levels in depressed patients are lower than those of healthy controls and the majority of studies reported increases of BDNF levels after antidepressant treatment (antidepressant pharmacotherapy, electroconvulsive therapy). Sleep deprivation (SD) and also partial sleep deprivation (PSD) are effective for patients with antidepressant medication-resistant depression. However, the underlying molecular mechanisms of SD is not well understood especially those explaining the rapid, but transient, antidepressant effect of SD.

Methods: Twenty-eight patients suffering from MDD, which were naïve to sleep deprivation therapy, participated in this study. Participants experienced PSD with a supplementary mono-therapy of mirtazapine. In addition, the stimulant modafinil or placebo was applied starting during PSD. PSD-response was assessed by 6 items of the *Hamilton Depression Rating Scale* (HDRS) before (day 0), during and after PSD (day 1) and at follow-up after two weeks (FU2). For serum sampling blood was obtained at seven different time points: at 8am (t1), 2pm (t2) and 8pm (t3) for baseline (day 0), at 1.30am (t4) during PSD, as well as 8am (t5), 2pm (t6) and 8pm (t7) after PSD (day 1).

Results: Notably, responders showed a significant diurnal BDNF serum variation the day before PSD, relative to non-responders. Similarly, responders showed a diurnal BDNF serum profile also after PSD in contrast to non-responders. PSD induced a fast increase in BDNF serum levels, descriptively, within a time frame of several hours at the day after PSD which parallels clinical findings. Accordingly, BDNF serum levels increased with decreasing depression scores in all participants. Adjuvant modafinil medication was not related to changes in BDNF serum levels.

Conclusions: Our results indicate that the elasticity in diurnal serum BDNF variation is associated with favourable treatment response to PSD in patients suffering from MDD. Therefore, a normalized BDNF serum profile which oscillates in a circadian fashion seemed to precede, rather than follow a

favourable treatment outcome in depressed patients. Thus, we suggest that diurnal profiling of BDNF should be monitored at baseline especially before therapeutic intervention starts for the purpose of early response prediction.

INTRODUCTION

There is growing evidence that both the pathophysiology and the treatment of major depressive disorder (MDD) are linked to alterations of neurotrophic factor expression such as brain-derived neurotrophic factor (BDNF) in various brain regions, including the hippocampus [1,2,3]. These can involve reversible changes, which explain the link between depressive episodes and subsequent recovery with neuronal plasticity. Exposure to stress, which is related with, but not required for the onset of MDD [4] in humans, advances or worsens depressive episodes [5]. Preclinical and clinical studies demonstrate that reductions of the total volume of neurons and neuronal loss occur in stress and depression in the adult hippocampus [6]. These hippocampal alterations can be reversed by chronic antidepressant treatment [6].

The stress response leads to an increase in glucocorticoids via activation of the hypothalamic–pituitary–adrenal (HPA) axis. However, under chronic stress the HPA axis regulation may become deregulated. Such HPA axis alterations during an acute depressive episode and its normalization after successful treatment are the most consistently observed laboratory findings in patients with MDD [7,8,9]. In rodents it could be already shown that acute and chronic stress decreased levels of BDNF expression in the dentate gyrus and the hippocampus. This reduction seems to be mediated partly via stress-induced glucocorticoids [10].

BDNF has received much attention among candidate downstream effectors involved in antidepressant-mediated hippocampal neurogenesis [11]. Indeed, chronic antidepressant treatment seemed to be associated with an increased BDNF expression in the hippocampus [2]; likewise, a direct infusion of BDNF into the hippocampus was sufficient to produce an antidepressant-like action in mouse models of depression [12]. Taken together, multiple neurobiological mechanisms seem to be involved in mediating the therapeutic effects of antidepressant therapy. Some of these mechanisms seem to play a role for neuroprotection and neurogenesis. The complexity of these mechanisms could explain the fact of slow onset of action in antidepressant treatments [13,14,15].

Neurotrophic functions of BDNF are connected to neuronal survival, memory, learning, appetite and sleep [16,17], leading to the neurotrophin hypothesis which is based on these features and claims that stress-related mental disorders result from stress-induced decreases in BDNF expression [18]. In sum the “neurotrophic hypothesis of depression” proposes that depression is associated with reduced brain BDNF levels and that successful antidepressant treatment is mediated by an increase in BDNF levels [18,19]. However, revealing the cellular and molecular pathways underlying the action of antidepressants remains an extremely challenging mission.

Circulating BDNF is found in both human serum and plasma and a large amount is stored in human platelets [20]. However, the source of peripheral BDNF is not clear yet. Since the protein can cross the blood brain-barrier in both directions, circulating BDNF correspondingly correlates with cortical BDNF concentrations [21] suggesting peripheral BDNF as a promising candidate biomarker in the association with depression and antidepressant treatment response. Several studies showed that serum BDNF levels in drug-free MDD patients were significantly lower compared to healthy subjects [22,23,24,25]. Other studies reported that plasma BDNF levels were lower in drug-free MDD patients [24,26]. Some clinical studies have evaluated the changes of plasma or serum BDNF levels before and after antidepressant treatments among MDD patients and most studies reported increases of BDNF levels after antidepressant treatment [22,27,28,29,30], although very recent data indicate that the increase in serum levels of BDNF during antidepressant treatment appears to be confined to some but not all antidepressants [31]. Moreover, some studies provide important evidence that an antidepressant-induced increase in BDNF levels is more prominent in responders than non-responders [28,32] or even exclusively restricted to responders [32].

Furthermore, different antidepressant strategies including antidepressant drugs, electroconvulsive therapy (ECT) and physical exercise were associated with an increase of peripheral BDNF levels [33,34]. Sleep deprivation (SD) is clinically well documented, robust, and fast-acting method for the treatment of severely depressed patients [35]. Beside SD of the total night, partial sleep deprivation (PSD) in the second half of the night has also shown comparable antidepressant effects in patients with major depression [35]. However, the underlying molecular and cellular mechanisms of SD are not well understood, especially those explaining the rapid, although transient, antidepressant effect of SD, since a relapse into depression occurs in most patients following the recovery night [36]. Recent evidence indicates that an up-regulation of *Bdnf* gene expression after SD in rats might be relevant for its mode of action [33,37]. Moreover, evidence also supports a role for BDNF in the modulation and mediation of circadian rhythms which seem to be disturbed in mood disorders. Interestingly, the presence of a diurnal BDNF rhythm was also recently demonstrated in healthy humans where plasma BDNF displayed highest concentrations in the morning, followed by a substantial decrease throughout the day, and the lowest values at midnight [38]. However, diurnal variation of blood BDNF was neglected until now in studies investigating MDD patients.

In the present study, we wanted firstly to explore the effect of partial sleep deprivation (PSD) as an alternative antidepressant intervention on serum BDNF levels within an acute time window of 12 hours during day after PSD night in MDD patients. Secondly, we investigated whether treatment with modafinil, which seems to be able to reduce micro sleep during SD thereby stabilizing the treatment response to PSD when compared with placebo [36], has an impact on serum BDNF levels after PSD. Due to the fact that BNDF levels oscillate in the periphery in a circadian fashion, we thirdly studied diurnal variation of serum BDNF levels before and after PSD and whether serum BDNF profile is altered in response to PSD. Finally, we wanted to investigate whether baseline BDNF levels before

PSD and treatment-associated changes are related to clinical and depressive symptoms and/or treatment response. Thus, this study was specifically designed to gain for the first time results on daily peripheral BDNF profiles in MDD patients and to explore the hypothesis if a diurnal BDNF pattern is associated with prediction of antidepressant therapy response already at a very early stage of intervention, in this case within hours. This is in contrast to all other published studies where treatment related BDNF changes were collected and analysed not earlier than after one up to six weeks of antidepressive therapy.

MATERIAL AND METHODS

Subjects and study design

Twenty-eight in- and out-patients (13 men, 15 women, age 45.1 ± 12.1 years; range 19–65 years) with the diagnosis of major depression (DSM-IV and ICD-10) participated in this study as previously published by Beck et al., 2010 [36]. For study design details, inclusion and exclusion criteria see Beck et al., 2010 [36]. In brief, all participants were naïve to sleep deprivation therapy and randomly assigned to either additional modafinil or placebo treatment. Inclusion criteria comprised a HDRS-score of ≥15 and the presence of significant daytime sleepiness. PSD-response was assessed by 6 items of the *Hamilton Depression Rating Scale* covering the items depressive mood, feelings of guilt, working and leisure activities, depressive inhibition, psychological symptoms of anxiety, somatic symptoms [39]. An improvement of at least 30% from baseline was required to determine PSD response. The modafinil group consisted of eight men and six women, age 46.2 ± 12.2 years (n=14, HDRS 21 item before PSD 21.71 ± 6.56 and at day after PSD 15.71 ± 9.12; 6 item before PSD 9.43 ± 2.79 and at day after PSD 6.50 ± 3.69) and the placebo group of five men and nine women, age 43.9 ± 12.2 years (n=14, HDRS 21 item before PSD 21.79 ± 4.72 and at day after PSD 12.64 ± 8.38; 6 item before PSD 8.71 ± 2.40 and at day after PSD 5.07 ± 3.85; values of all ratings were not different compared to modafinil) [36]. According to ethical considerations, all patients were treated with a monotherapy of 30mg mirtazapine daily at 9pm throughout the study (Figure 1).

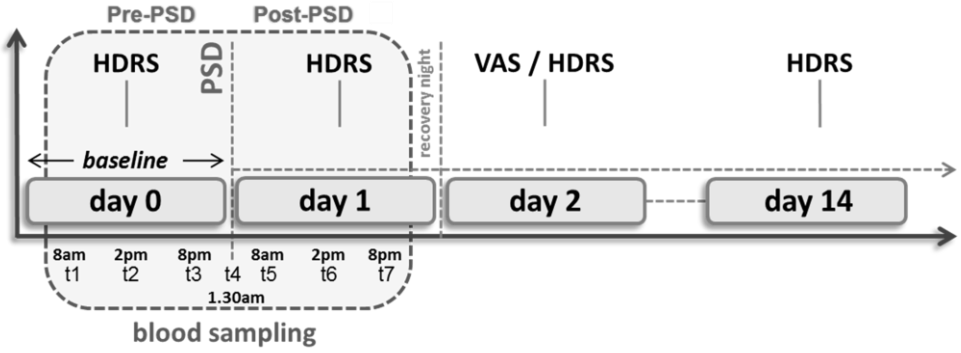

Figure 1. **Study design and serum sampling.** Blood was drawn at day 0 (baseline) before PSD at 8am (t1), 2pm (t2), 8pm (t3) and at day 1 at 1.30am (t4), 8am (t5), 2pm (t6) and 8pm (t7). Placebo controlled additional morning treatment with the stimulant modafinil started during PSD and maintained over 14 days. Depression severity using the *Hamilton Depression Rating Scale* (HDRS) together with mood, tiredness and relaxation were assessed with *Visual Analogue Scales* (VASs) for psychological functioning at days 0, 1 and 2 ("after recovery night") as well as after two weeks (day 14, FU2) of on-going treatment.

Compared to placebo, modafinil was efficient in reducing daytime microsleep following partial sleep deprivation but did not enhance the antidepressive effects of PSD and did not stabilize antidepressive effects over two weeks [35].

Serum sampling

For serum sampling blood was obtained in a serum separator tube from the antecubital vein at seven different time points. Day 0 (before PSD) at 8am (t1), 2pm (t2) and 8pm (t3) for baseline and day 1 at 1.30am (t4) during PSD, 8am (t5), 2pm (t6) and 8pm (t7) after PSD (Figure 1). After 30min of clotting time, whole blood was centrifuged at 1000xg for 30min to separate the serum. Serum was collected in aliquots to avoid several freezing cycles and kept at -80°C before assaying.

Measurement of serum BDNF levels

Serum BDNF levels were assessed with an enzyme-linked immunoabsorbant assay (ELISA) kit (Promega BDNF Emax®, Madison, Wis., United States). Samples were appropriately diluted (between 1:100-1:150) and detection of total soluble BDNF was carried out in an antibody sandwich format like described in the manufacturers protocol. The absorbance was measured within 30min in a microplate reader set at 450nm to determine BDNF concentrations according to the standard curve. All assays were carried out in duplicates and means were calculated.

Behavioural ratings

Severity of depression was evaluated by the 21 Item version of *Hamilton Depression Rating Scale* (HDRS; Hamilton, 1967) at day 0 before, day 1 after PSD, following the recovery night at day 2 and after 2 weeks (FU2). PSD-response was assessed by 6 items of the HDRS covering the items depressive mood, feelings of guilt, working and leisure activities, depressive inhibition, psychological symptoms of anxiety and somatic symptoms [39]. An improvement of at least 30% from baseline was required to determine PSD response according to [40].

In addition participants were asked to rate their experienced level of relaxation at day 2 after recovery night on a *Visual Analogue Scale* (VAS) consisting of a horizontal line, 100mm in length, ranging from „not at all relaxed'' at the left end to „extremely relaxed'' at the right end. Participants marked on the line the point that represents their perception of the current state. The VAS score could range from 0 to 10 and was determined by measuring in centimetres (with millimetre accuracy) the distance from the left hand end of the line to the point that the participant marked.

Questionnaires to assess potential confounders

Besides a sociodemographic questionnaire to assess sex, age and BMI, participants had to indicate substance consumption, which could interfere with biological analyses.

Statistical Analysis

Analysis of variance (ANOVA) was used for multiple group parametric comparisons. Associations between variables were computed with Pearson`s correlations. To compare the effect size Cohen`s d was calculated to compare serum BDNF values among subjects, responders and non-responders. The level of significance was set at $p \leq 0.05$. Preliminary calculations revealed that BDNF serum levels were not associated with sex and age ($rs < .20xx$, $p > .35$). Moreover, BDNF serum levels did not statistically significantly differ between smokers and non-smokers (all $ts<1.56$, $p>0.13$). As a result, sex, age and smoking were not entered as co-variates.

RESULTS

Daily serum BDNF profile from MDD patients before and after PSD

At baseline (day 0), a diurnal variation pattern could be detected in serum BDNF levels from all participants (n=28) which decreased during the day starting with the highest concentration in the morning until BDNF concentrations reached lowest level in the night before PSD (Figure 2A). After PSD, descriptively though not statistically, BDNF levels were increased of 10.4% (at 8am), 16.2% (at 2pm) and 12.3% (at 8pm) respectively, relative to the corresponding BDNF levels of day 0 (Figure 2B).

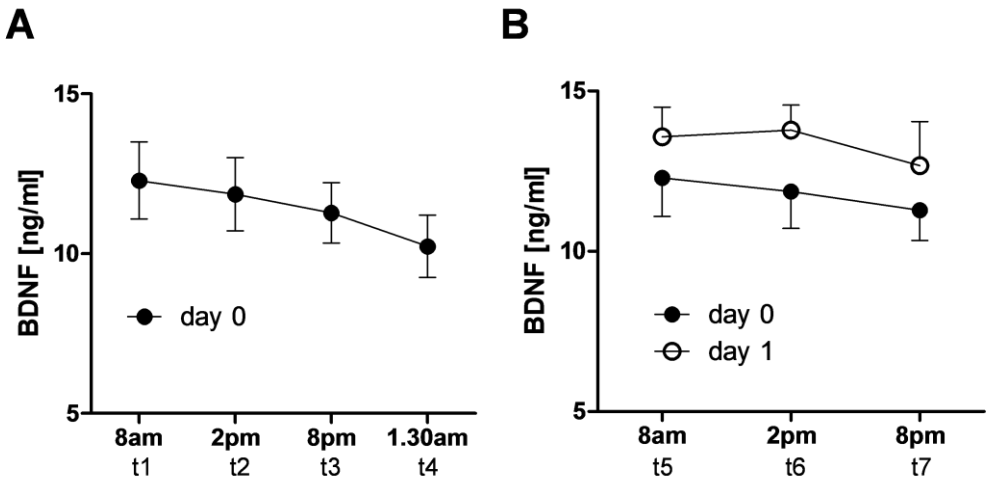

Figure 2. Daily variation of serum BDNF levels. (A) Baseline serum BDNF levels (means ± SEM) decreased during the day starting with the highest concentration in the morning (8am, t1) until they reached lowest level after midnight (1.30am, t4) in all patients. (B) After PSD serum BDNF levels (means ± SEM) were elevated throughout day 1, relative to levels at baseline (day 0) of 10.4% at 8am, 16.2% at 2pm and 12.3% at 8pm. This difference in BDNF levels between baseline and day 1 was not statistically significant, but rather describes a trend of serum BDNF levels to increase after PSD.

No effect of additional treatment with modafinil on BDNF levels

To evaluate the effect of additional modafnil treatment starting at 1.30am during PSD we compared serum BDNF levels of day 1 between patients with modafinil and placebo treatment. A series of Chi-square tests revealed no statistically significant associations between treatment condition and response

(response day 1: $X^2(1, N=25)=0.24$, $p=0.62$; response day 2: $X^2(1, N=25)=0.05$, $p=0.82$; response FU1 $X^2(1, N=25)=0.27$, $p=0.87$; response FU2: $X^2(1, N=24)=1.34$, $p=0.27$).

Treatment response and daily changes in serum BDNf levels

BDNF and acute improvement on HDRS-6

To assess whether this diurnal BDNF pattern was associated with an antidepressant treatment response to PSD we focused primarily on serum BDNF levels of day 0 before PSD with corresponding depression severity ratings after the intervention. Depressive patients who showed an acute improvement on the HDRS-6 after PSD on day 1 compared to day 0 exhibited a prominent diurnal pattern of serum BDNF levels at baseline (during the day 0 before sleep deprivation) (Figure 3).

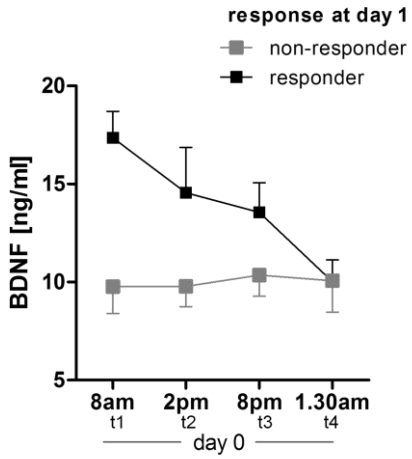

Figure 3. Baseline changes of serum BDNF levels. BDNF levels (means ± SEM) were significantly elevated in patients who respond to PSD at day 1 relative to non-responders at 8am, t1 ($t=3.370$, $p=0.003$). Effect size calculations indicated that higher BDNF levels were prominent in day 1 responders at 8am, t1 ($d=1.57$), 2pm, t2 ($d=0.78$) and 8pm, t3 ($d=0.57$) at baseline. ANOVA calculation revealed a significant group effect between responders and non-responders at day 1 for baseline serum BDNF levels before PSD ($F(1.87)=13.88$; $p=0.003$).

Serum BDNF levels were significantly elevated in responders (identified as acute responders on day 1) relative to non-responders at 8am (Figure 3) of both days (BDNF values at 8am: day 0, $t=3.370$, $p=0.003$; day 1, $t=2.356$, $p=0.028$; *data not shown*). In addition, acute responders at day 1 had descriptively higher BDNF levels at 2pm and 8pm at baseline (day 0: t2 and t3). Moreover, effect size calculations revealed that higher BDNF levels were prominent in acute responders relative to non-responders at 8am (t1) ($d=1.57$), 2pm (t2) ($d=0.78$), 8pm (t3) ($d=0.57$) of day 0 (Figure 3). Of note, there was no diurnal pattern of serum BDNF observable before the intervention in patients identified as acute non-responders on day 1, which is reflected by a significant group effect between BDNF values of acute responders and non-responders at day 0 before PSD ($F(1.87)=13.88$; $p=0.003$).

After recovery night, responders at day 2 (identified as responders on day 2) revealed descriptively higher BDNF levels relative to non-responders (*data not shown*). Effect size calculations emphasized increased BDNF levels in responders compared to non-responders at 8am (t1) ($d=0.76$), 2pm (t2) ($d=0.67$) at day 0 and 2pm (t6) ($d=0.53$) of day 1.

BDNF and long-term improvement on HDRS-6

In a further step we explored to which extent pre- and post-PSD serum BDNF levels of day 0 and 1 exhibited diurnal pattern characteristics for long-term responders and non-responders (long-term

HDRS-6 ratings after two weeks [FU2]) (Figure 4A). Again, BDNF-levels at baseline were descriptively higher in long-term responders at 8am (day0, t1) compared to non-responders. Effect size calculations emphasized higher BDNF levels in FU2-responders at 8am (t1) (*d*=0.91), 2pm (t2) (*d*=0.71) of day 0, relative to non-responders (Figure 4A).

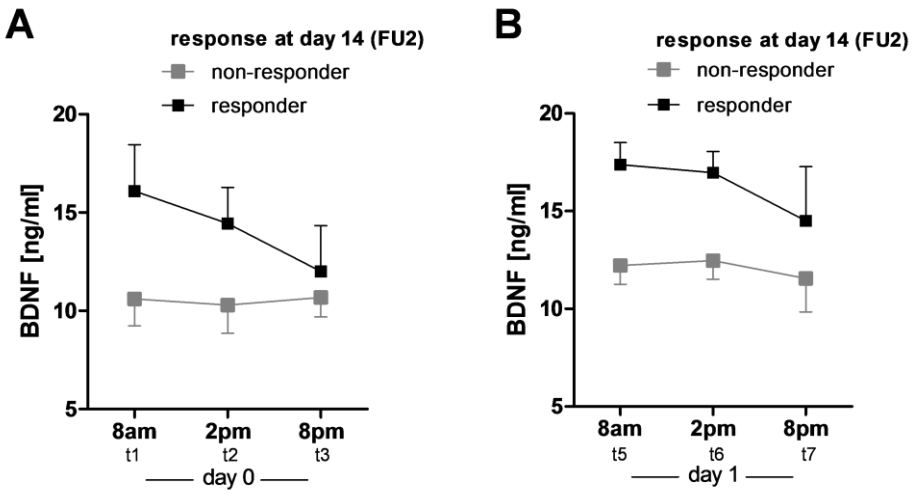

Figure 4. Baseline and day 1 changes of serum BDNF levels. (A) BDNF levels (means ± SEM) were descriptively higher in patients who responded after 14 days of on-going treatment relative to non-responders at baseline. Effect size calculations indicated that higher BDNF levels were prominent in day 14 responders at 8am, t1 (*d*=0.91) and 2pm, t2 (*d*=0.71). (B) There was a prominent increase in serum BDNF after PSD in day 14 responders. Serum BDNF levels were significantly elevated at day 1 for 8am, t5 (*t*=2.993, *p*=0.007) and 2pm, t6 (*t*=2.846, *p*=0.010) compared to non-responders.

In addition, there was a prominent daily change of serum BDNF after PSD in long-term responders. Thus, FU2 responders showed significantly higher serum BDNF levels after PSD on day 1 at 8am (t5) (*t*=2.993, *p*=0.007) and 2pm (t6) (*t*=2.846, *p*=0.010) compared to non-responders (Figure 4B). Furthermore, effect size calculations emphasized higher BDNF levels in FU2-responders at 8am (t5) (*d*=1.54) and 2pm (t6) (*d*=1.37) of day 1, relative to non-responders.

Importantly, there was again no diurnal baseline BDNF pattern observable for patients specified as non-responders after 2 weeks (Figure 4A). However, after PSD at day 1 a trend to a slight diurnal BDNF change was recognizable (Figure 4B), which was clearly less pronounced compared to responders.

Relationship between HDRS-6 improvement and treatment-related serum BDNF levels

After PSD, descriptively though not statistically, BDNF levels were increased in responders of 8.2% (at 8am), 17.5% (at 2pm) and 20.8% (at 8pm) as well as in non-responders of 15.2% (at 8am), 21.2% (at 2pm) and 8.2% (at 8pm) respectively, relative to the corresponding BDNF levels of day 0 (Figure 4B compared to 4A). The overall increase of serum BDNF on day 1 after PSD was prominent in responders as well as in non-responders at 2pm (Figure 4A, B). In agreement with other findings using different antidepressive treatment approaches [41,42], the HDRS-6 improvement 2 weeks after PSD

was significantly correlated with an increase of serum BDNF levels in all patients (r_p=-0.462; p=0.018) (Figure 5).

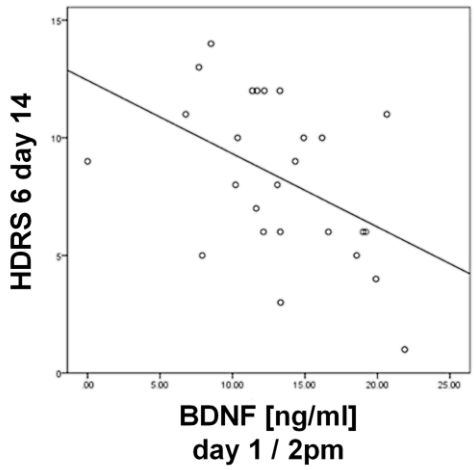

Figure 5. HDRS-6 improvement and peak serum BDNF levels at day 1. Lower HDRS-6 response scores at day 14 (FU2), indicating an improvement of depressive symptoms, significantly correlated with an increase in serum BDNF levels at day 1, 2pm (t6) in all patients (r_p=-0.462; p=0.018).

Relationship between psychological functioning and treatment-related serum BDNF levels

To assess whether psychological functioning in general is linked with post-PSD BDNF serum levels we correlated VAS scores for relaxation and mood after recovery night from day 2 with BDNF peak levels of day 1 at 2pm. *The Visual Analogue Scale* (VAS) has a long history of use for the assessment of subjective mood, strain, and pain, and the validity and reliability of this method is generally accepted. Here we found a significant correlation between increased relaxation (r_p=-0.425, p= 0.03) or improved mood (r_p=0.510, p=0.008) and increased BDNF levels in all patients (Figure 6A, B).

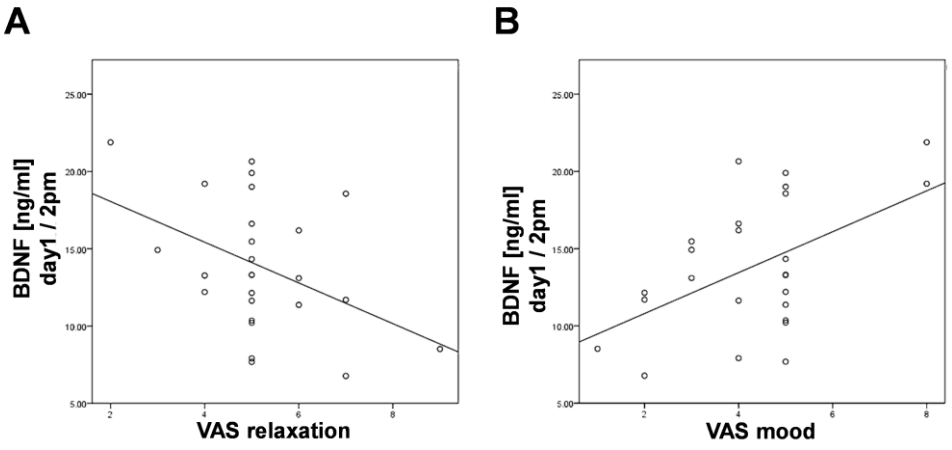

Figure 6. Psychological functioning is linked to peak serum BDNF levels at day 1. (A) Higher serum BDNF levels from day 1 at 2pm (t6) after PSD significantly correlated with increased relaxation expressed as lower VAS score values after the recovery night on day 2 (r_p=-0.425; p=0.03) in all patients. (B) Furthermore, after recovery night at day 2, improvement in mood expressed as higher VAS score values significantly correlated with higher serum BDNF levels from day 1 at 2pm (t6) after PSD (r_p=0.510; p=0.008) in all patients. (VAS) – Visual Analogue Scale

DISCUSSION

Key findings of the present study indicate that BDNF serum levels exhibited a significant diurnal variation among responders when compared to non-responders in a sample of patients suffering from major depressive disorder (MDD). Subjects identified as acute responders after partial sleep deprivation (PSD) at day 1 and even after 2 weeks of follow-up were associated with a daily change of serum BDNF at baseline before PSD. This variation of peripheral BDNF abundance revealed characteristics of a diurnal pattern since levels decreased during the day starting with the highest concentration in the morning, whereas non-responders did not exhibit diurnal BDNF variation in their serum. Thus, we can conclude that the presence of a diurnal serum BDNF profile in MDD patients predicts therapy response. Of note, there are only few data available on diurnal variation of blood BDNF levels in healthy subjects, but nothing is known from MDD patients in this regard.

Most biological functions are expressed in an oscillating manner within a 24-hour circadian period, regulated by endogenous biological clocks. A growing body of evidence also supports a role for BDNF and TrkB in the modulation and mediation of circadian rhythms. As a starting point, high levels of BDNF and TrkB expression were demonstrated in the rat suprachiasmatic nucleus (SCN) and hippocampus [43]. It was reported that BDNF protein and mRNA levels in the rat SCN showed evident signs of variation over the course of a circadian cycle. The SCN content of BDNF protein remained low throughout the subjective day, began to rise early in the subjective night, and reached peak levels near the middle of the subjective night [44]. Diurnal variation in BDNF protein expression levels was demonstrated in the cerebellum, hippocampus, and cerebral cortex [43,45]. Moreover, recognition that circadian rhythm disruption also plays a key role in mood disorders has led to the development of the new antidepressant agomelatine. Recent data from various groups showed that agomelatine led to an increase in BDNF expression in treated animals, and that this effect follows a specific temporal profile [46]. Interestingly, the presence of a diurnal BDNF rhythm was also recently demonstrated in healthy humans (only males were included in the study) where plasma BDNF displayed highest concentrations in the morning (8am), followed by a substantial decrease throughout the day, and the lowest values at midnight [38]. A similar circadian fluctuation in plasma BDNF levels was also found in women [47], even if the amplitude of the variation in BDNF levels appeared to be influenced by ovarian function with a blunted diurnal rhythm in the luteal phase. In the present study, we could confirm parallel changes in diurnal variation in serum BDNF in patients identified as responders of both gender. Thus, all the findings together emphasize the importance of the presence of a circadian BDNF rhythm in human health and wellbeing, while the absence seems to have a negative impact on successful treatment outcome in MDD.

The decline in peripheral BDNF levels during the day may be ascribed to a circadian secretory model on the one hand. Thus, it can be speculated that BDNF is secreted with a pulsatory circadian rhythm that is characterized by a progressive reduction in the amplitude of pulses throughout the day. On the other hand, it has been shown that brain BDNF is able to cross the blood–brain barrier via a rapidly

saturable transport system [48]. While brain BDNF peaks around midnight and thus may exert its modulating effect on neuroplasticity and long-term potentiation [44], peripheral BDNF in the blood, namely, both of plasma and serum, peaks in the morning at 8am and exhibits its lowest expression level at midnight, indicating that BDNF cycles in opposite phases in brain and blood. Why there is a discrepancy in temporal expression profiles between blood and brain is not clear, but might be due to a rapid transition from brain to blood with a time delay of about 12 hours or might reflect the endogenous circadian rhythm of BDNF in the periphery which oscillates in anti-phase to that of the brain. Importantly, this repeatedly reproduced result is in line with findings of Sartorius and colleagues, which investigated correlations and differences between serum and brain tissue BDNF levels after ECT in rats [49]. They demonstrated a positive correlation between brain and serum BDNF concentrations providing evidence that it can be justified to measure serum BDNF levels but only by consideration of a time delay to monitor brain tissue alterations in the periphery.

Ultimately, it is not clear to which extend diurnal changes of blood BDNF are related to a circadian secretory model or sex hormones next to malfunctions in the hypothalamic-pituitary-adrenal (HPA) axis regulation, different activity during the day and other environmental factors.

Moreover, the present study demonstrated that diurnal BDNF serum variations were not changed by the adjuvant medication with modafinil as a stimulant during PSD, which is consistent with our previous finding showing that modafinil was not able to enhance the antidepressive effects of PSD and did not stabilize antidepressive effects over two weeks in these patients [36]. In addition, PSD itself had also no significant impact on diurnal serum BDNF profile. This further emphasizes the importance of the presence of a daily BDNF profile in a circadian fashion at baseline with regard to therapy response prediction and is in line with data from Wolkowitz and colleagues [50] who also found that serum BDNF levels (only measured at the single time point 10am) before treatment with SSRI (escitalopram or sertraline) predicted SSRI response in depressed patients and responders to treatment exhibited higher pre-treatment BDNF levels than did non-responders. Thus, one can speculate that diurnal BNDF variation represents a prerequisite for successful therapy response independent from the specific treatment strategy, e.g. antidepressants drugs or SD.

Furthermore, our findings demonstrated that post-PSD BDNF serum levels were higher in acute (HDRS-6 ratings at day 1) as well as long-term (HDRS-6 ratings after two weeks) responders to treatment ($\geq$ 30% improvement in depression ratings) when compared to non-responders. Consistently, increased BDNF serum levels (peak levels at 2pm at day 1) were associated with decreased depressive symptoms in all patients. These data are in line with other studies also providing evidence that an antidepressant-induced increase in BDNF levels is more prominent in responders than non-responders [42] or even exclusively restricted to responders [32], while the early non-increase in serum BDNF levels predicted failure of antidepressant treatment (different classes of antidepressants) in patients with major depression [51]. Notably, beyond the association of increased BNDF with decreased depressive symptoms, our results indicate an improved emotional state of mind and greater relaxation

related to elevated serum BDNF levels further confirming a normalizing effect on the deregulated relationship between BDNF, stress and HPA axis system in MDD.

In the present study, post-treatment BDNF levels were investigated already at an very early stage of intervention, in this case within hours after PSD (up to 18 hours by using a six hour interval post PSD, namely at 8am, 2pm, and 8pm). This is in contrast to all other published studies where treatment related BDNF changes were collected and analysed not earlier than after one up to six weeks of antidepressive therapy. The rapid effect of PSD on BDNF levels, however, might be related to the rapid antidepressant effect of PSD at day 1 and a relapse into depression occurs in most patients following the recovery night (already at day 2) [36]. In agreement with our findings, others have consistently shown that prolonged wakefulness as a result of sleep deprivation, which can be considered as a stressor for the brain, leads to an increase in BDNF [33], while sleep disturbance was linked to reduction of BDNF levels [52], confirming a bidirectional stress model with which both can be explained, chronic stress induces a deregulation of the HPA system leading in the long term to sleep disturbance and decreased BDNF levels, whereas acute sleep deprivation, e.g. PSD, can be used as therapeutic intervention in some insomniac or depressed patients as a compensatory process to normalize BDNF levels.

In summary, the present study adds very important new evidence to the currently existing BDNF literature dealing with MDD: i) an a-priori diurnal BDNF serum level variation precedes a more favourable treatment outcome in depressive patients, and ii) PSD may exert a very rapid effect on serum BDNF levels within a time frame of several hours at the day after PSD which parallels clinical findings.

Thus, we suggest that next to analyses of pre- and post-treatment serum BDNF levels collected at a single time point per day, diurnal proof of peripheral BDNF should be monitored at baseline especially before intervention starts for the purpose of therapy response prediction. Moreover, the results of our study might provide a valuable model for the investigation of biomarkers in the treatment of MDD and other psychiatric disorders and might encourage research in the area of early predictors of therapy response in order to shorten the duration of psychopharmacological treatment until the determination of insufficient effectiveness. Further studies with a larger number of patients and different treatment strategies should be conducted to verify the results of our exploratory investigation and to elucidate the underlying mechanisms more closely, especially the role of the stress hormone system.

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C. Discussion

Stress-related mood disorders like major depressive disorder (MDD) are complex, multivariate and imply numerous neuronal substrates and brain regions. Currently available antidepressants acutely increase monoamine levels but the chronic treatment is required for the onset of antidepressant effect, meaning that enhanced serotonergic and/or noradrenergic neurotransmission is not the only main trigger factor for clinical actions of these drugs.

In the three present studies we investigated serum BDNF levels in the context of stress-related disorders, especially MDD. In recent decades, BDNF became more and more a key molecule in the field of biomarkers and/or target research for treatment of mood disorders, since there is increasing evidence suggesting a role in the pathophysiology of mood disorders (Shimizu, Hashimoto et al. 2003). Of note, BDNF alone may not be sufficient to explain the pathology of stress-related mood disorders, but it remains an important risk factor for the development. Furthermore, stress-related disorders are often accompanied with sleep related problems and sleep is an important component of physical and mental health (Van Dongen and Dinges 2003; Stein, Belik et al. 2008; Walker 2009). Therefore, our research focussed also on disturbed sleep wake regulation, namely insomnia, besides MDD. Of note, our results showed that serum BDNF levels significantly correlated with severity of insomnia and that sleep-disturbed subjects revealed reduced serum BDNF levels (Giese, Unternahrer et al. 2013) in the setting of this explorative study. The significance of our preliminary data was further emphasized when we assessed an external control group that had recovered form occupational burnout. Again, serum BDNF levels were significantly lower in those subjects reporting symptoms of fatigue compared to sleep healthy subjects. This was in line with a significant negative correlation of serum BDNF levels with symptoms of tiredness and fatigue. These findings strongly support the hypothesis of increased stress vulnerability due to sleep loss, in conjunction with decreased serum BDNF levels. This could be explained from two points of view. First, decreased serum BDNF levels could reflect impaired BDNF concentrations in the CNS, which contribute to hippocampal atrophy, like it is repeatedly documented for MDD (Stockmeier, Mahajan et al. 2004). Since the hippocampus provides an important source of negative modulation of the HPA stress hormone axis through its projection to the hypothalamus, hippocampal dysfunction could contribute to the deregulation of the stress response system, which ends up in higher stress vulnerability. Secondly, it is known that perturbed sleep behaviour is common in many stress-related disorders. Our results revealed that sleep behaviour is linked to BDNF (Giese, Unternahrer et al. 2013) and therefore sleep may be a key moderator for stress perception. Meaning that, impaired sleep wake regulation could affect the stress response system, leading to a malfunction of the negative feedback regulation of the HPA system and increased stress vulnerability. Therefore, increased glucocorticoid levels may act on BDNF expression (Smith, Makino et al. 1995; Schaaf, de Jong et al. 1998) via the transcription factor CREB and supress BDNF expression, namely BDNF mRNA. In animal models related to stress (Russo-Neustadt, Ha et

al. 2001) it has already been shown that BDNF mRNA levels were significantly decreased. However, the link between stress and sleep is missing. Nevertheless, both approaches are in line with a possible explanation that parallel BDNF changes occur in the brain and serum of depressive patients (Shimizu, Hashimoto et al. 2003). It was already reported that brain and serum BDNF levels underwent similar changes during maturation and aging processes in rats (Karege, Schwald et al. 2002) and that serum and cortical BDNF levels are positively correlated (Karege, Schwald et al. 2002). Still, a major question, which is not solved yet, is whether low BDNF levels are primary or secondary in stress-related disorders. Primary reduced BDNF levels might reflect a genetic vulnerability and secondary reduced BDNF levels might explain the hypothesis of an acquired biological vulnerability. From the perspective of stress-induced BDNF reduction, like discussed above, decreased BDNF levels may reflect a collapse of the stress-adaptation system and its failure to protect the brain from stress-induced neuronal degeneration. A possible link in this open question might be sleep, acting as a moderator between stress perception and adaptation of BDNF. So far there are no studies, which investigated the interplay of stress, sleep and BDNF.

Therefore, we hypothesized a possible effect of stress on the previously identified relationship of sleep and BDNF levels in a further study. Our results show that the severity of sleep impairment is positively correlated with stress experience. Interestingly, this is only found in subjects without insomnia, which highlights the importance of sleep and sleep quality in terms of stress load and perception. If sleep is maintained or disturbed might explain why some individuals are able to handle a certain stress load while others develop a mental disorder. Indeed, we identified that sleep mediates the association between stress and sleep (manuscript submitted), which could explain that increased stress negatively affects sleep and in turn affects BDNF levels. Therefore, our results extend the finding of stress-induced BDNF decrease with the suggestion of sleep as a possible key mediator. It is likely that the interplay of chronic sleep impairment and in turn increased stress levels, reflected by decreased BDNF levels, is an essential mechanism in the pathology of stress-associated mood disorders.

This would be in line with the idea that not only the balance of certain neurotransmitter, namely monoamines, is crucial to develop a mood disorder, but also adequate BDNF levels (Castren 2005) promoting neuronal plasticity, which provides a factor supporting mental health. Of course, there might be additional factors influencing mental health with regard to BDNF and the stress response system. However, BDNF seems to play an important role in the activity-dependent information processing of the brain and the body, rather than just the balance of distinct signalling molecules. Next to appropriate neuronal communication restricted by BDNF levels reflecting a healthy range, we could show in a further study that not only single endpoint levels *per se* at a certain time point during the day but rather a daily variation profile shed light on the adaptive process regarding BDNF. Notably, we could show that the diurnal variation profile of serum BDNF is an important factor regarding prediction of antidepressant therapy response. Moreover, our results showed that the antidepressant therapy intervention of the present study, namely partial sleep deprivation (PSD) (manuscript

submitted), is followed by a fast increase of serum BDNF levels within hours. This is in contrast to most studies assessing BDNF with regard to antidepressant therapy response after four to six weeks, while overall BDNF levels increased within 24 hours between 10-16 % already after PSD in the present study. However, most importantly, subjects who were identified as responders directly after PSD night, revealed a prominent diurnal variation of serum BDNF levels during baseline day before spending half of the night awake. This finding was also true for responders after 14 days, but could not be shown for non-responders, which exhibited a more or less flat line of serum BDNF levels throughout the baseline day. Furthermore, at the day after sleep deprivation, the diurnal variation of BDNF was even more prominent reflected by increased levels per daily time point and a distinctive diurnal curve. In addition, increased BDNF levels after PSD were associated with improvement of depressive symptoms in MDD patients. Hence, our results support the hypothesis that a diurnal variation throughout the day seems to be important for therapy response at least for the therapy intervention settings in the present study. But this could explain additionally, like discussed above, why people react differently regarding to certain stressors. Sleep deprivation for one or half of the night as an antidepressant therapy intervention represents an acute stressor for body and brain. As compensatory response BDNF increases to normalize disturbed serum BDNF levels. Interestingly, this prolonged wakefulness was only associated with a response to therapy intervention, when patients exhibited a prominent daily variation of serum BDNF levels at baseline. When this variation throughout the day was absent, patients did not respond to partial sleep deprivation. It is possible, that an undisturbed circadian regulation of BDNF levels may be responsible for proper adaptation to external stressors and therefore maintaining necessary information processing supporting mental health. This phenomenon is already known from glucocorticoids, namely cortisol, which is under circadian regulation throughout the day (Weitzman, Fukushima et al. 1971; Smyth, Ockenfels et al. 1997).

In sum, this thesis reports an association of sleep with BDNF and that stress-induced BDNF modifications may be modulated by sleep. Furthermore, a diurnal variation of serum BDNF levels seems to play an important role in antidepressant therapy response and prediction. Our findings support the role of BDNF in adaptation to mood-influencing stressors that could lead to the development of depression. This adaption processes may be responsible for different aspects of mood regulation and antidepressant-like effect. However, normalization of BDNF during antidepressant treatment appears to be confined to some but not all antidepressants and does not correspond with amelioration of clinical symptoms (Molendijk, Bus et al. 2011). This could be explained with changes in central BDNF concentrations, following reduced insomnia rather than depressive symptoms and antidepressants differentially influencing sleep. It is also important to mention, that antidepressants differentially influence sleep and therefore sleep wake regulation, specifically insomnia, has to be carefully controlled, as well as improvements of sleep during therapy. Possibly there is a unique and

bidirectional pathway from illness to recovery that implicates BDNF and synaptic plasticity. However, it is still a matter of discussion if the effect of antidepressants on neurogenesis involves always neurotrophins. Thus, our preliminary findings suggest that serum BDNF levels are not associated with a specific (categorical) diagnosis, but may be associated with insomnia symptoms independent of diagnosis (dimensional). In line with this, we suggest that, when analysing serum BDNF levels in depressed patients, insomnia symptoms should be carefully controlled, as well as improvements in sleep during therapy.

If stress in general has such a pervasive long-term effect on the brain as well as on the whole body there should be some ways to reduce the negative consequences. From the standpoint of the individual, it seems obvious that a major goal should be to try to improve sleep quality and quantity as well as stress coping strategies, to have a good social support and a positive outlook on life, to have positive self-esteem, to maintain a healthy diet, to avoid smoking and to engage in regular moderate physical activity (McEwen 2008). In accordance with our data, one can speculate that the inconsistence of published data regarding BDNF in depression and therapy response might base on the following bias: (i) no monitoring of sleep improvement, only depressive symptomatology, (ii) no examination of the existence of a diurnal BDNF profile before therapy intervention and (iii) data for single point BDNF assessment after antidepressant therapy is quite variable – from four to eight weeks – therefore, it might be that an acute, transient increase of BDND levels is missed.

Further studies including the monitoring of insomnia, objective sleep measurements, such as sleep polysomnography, should be conducted to verify the results of our exploratory investigation and to clarify the underlying mechanisms more closely. Especially the underlying BDNF downstream signalling pathways and the relationship between BDNF and cortisol with their role to the stress hormone system have to be further elucidated. Moreover, the existence of a circadian profile of circulating BDNF should be examined, particularly before therapeutic intervention starts.

D. Abbreviations

Abeta	beta-amyloid protein
ACTH	adrenocorticotropin
AD	Alzheimer's disease
AKT	serine/threonin kinase
BDNF	brain-derived neurotrophic factor
Ca^{2+}	calcium
CaMKII	calcium-calmodulin dependent kinase
CDS	coding sequence
CPE	carboxypeptidase E
CREB	cAMP calcium response element binding protein
CRF	corticotropin-releasing factor
DG	dentate gyrus
DNA	deoxyribonucleic acid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders-IV
ERK	extracellular signal related kinase
Gab1	Grb-associated binder 1
GR	glucocorticoid receptor
Grb2	growth factor receptor bound protein 2
HPA	hypothalamic-pituitary-adrenal
IFN	Interferon
IFN-γ	Interferon γ
IL-10	Interleukin 10
IL-1β	Interleukin 1β
IL-2	Interleukin 2
IL-6	Interleukin 6
IRS1/2	insulin receptor substrates ½
kDa	kilo Dalton
LDCV	large dense core vesicles
MAOI	monoamine oxidase inhibitor
MAPK	mitogen-activated protein kinase
MD	Major Depression
MDD	major depressive disorder
MEK	MAP/Erk kinase
MR	mineralcorticoid receptor
mRNA	messenger RNA

NAc	nucleus accumbens
NGF	nerve growth factor
NT3	Neurotrophin-3
NT4	Neurotrophin-4
$p75^{NTR}$	pan neurotrophin receptor 75
PFC	prefrontal cortex
PI3K	phosphatidylinositol-3 kinase
PKC	protein kinase C
PLCγ	phospholipase-C-γ
PVN	paraventricular nucleus
Raf	Ras associated factor
Ras	GRP binding protein
RCT	randomised controlled trial
REM	rapid eye movement
RNA	ribonucleic acid
SD	sleep deprivation
Shc	Src homology domain containing
SNP	single nucleotide polymorphism
SNRI	selective noradrenaline reuptake inhibitor
SOS	son of sevenless
SSRI	selective serotonin reuptake inhibitor
SWA	slow wave activity
TNF- α	tumor necrosis factor α
TNF	tumour necrosis factor
Trk	tropomyosin-related tyrosine kinase
TrkA	tropomyosin-related tyrosine kinase receptor A
TrkB	tropomyosin-related tyrosine kinase receptor B
TrkC	tropomyosin-related tyrosine kinase receptor C
TSD	total sleep deprivation
UTR	untranslated region
VTA	ventral tegmental area
WHO	World Health Organization

E. Literature

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F. Curriculum Vitae

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Personal Data

date of birth:	January 20th, 1983
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Education

2009/01 – present	**PhD student** at the Neurobiology Laboratory for Brain Aging and Mental Health, Head Prof. A. Eckert, Transfaculty Research Platform, Molecular & Cognitive Neuroscience, University of Basel, Switzerland.
2008/04 – 2009/12	**Postgraduate semester** at the UZH, Zurich, Switzerland with main focus on molecular neuro-oncology.
2005/10 – 2007/09	**Master of Science** at the University of Osnabrück, Germany MSc programme "cellular biology" with main subjects: neurobiology, biophysics and developmental biology. Optional subjects: organic chemistry, biochemical aspects of clinical medicine. Master thesis at the Neurobiology Laboratory under the supervision of Prof. R. Brandt (Title: Interactions and competition of disease-relevant tau constructs).
2002/10 – 2005/09	**Bachelor of Science** at the University of Osnabrück, Germany BSc programme "cellular biology" with main subjects: neurobiology, genetic and microbiology. Bachelor thesis at the Neurobiology Laboratory under the supervision of Prof. R. Brandt (Title: Development of new methods for neuronal transfection).
1995/09 – 2002/07	**University-entrance diploma**, Ratsgymnasium Osnabrück, Germany.
1993/08 – 1995/07	**Qualification for secondary school**, Orientierungsstufe Schule am Roten Berg, Hasbergen, Germany.

Advanced Qualifications

11/2012	**Swiss Society for Neuroscience: SNG/SSN, SGBP/SSPB and SGVN/SSNC** Participation: joint meeting
10/2012	**European College of Neuropsychopharmacology (ECNP)** Participation: 25th ECNP congress
09/2012	**International Society of Psychoneuroendocrinology (ISPNE)** Participation: 41st annual ISPNE conference
10/2011	**Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP)** Participation: 27. Symposium der AGNP
09/2011	**European College of Neuropsychopharmacology (ECNP)** Participation: 24th ECNP Congress
03/2011	**Swiss Society for Neuroscience (SSN)** Participation: SSN Annual Meeting
06/2010	**Society for the Study of Fatty Acids and Lipids (ISSFAL)** Participation: 9th ISSFAL Conference
03/2010	**Swiss Society of Biological Psychiatry (SSBP)** Participation: 30. Jahresmeeting SSBP „Emotions – Nature and Nurture“
03/2010	**European College of Neuropsychopharmacology (ECNP)** Participation: 2010 ECNP Workshop on Neurophsychoparmacology for Young Scientists in Europe
06/2009	**Neuroscience Upper Rhine Network (Neurex)** Participation: Annual Meeting „Organizational principles in the nervous system: compartments & ensembles“
05/2009	**Neuroscience Upper Rhine Network (Neurex)** Participation: Meeting „Revisiting mitochondria`s functions and dysfunctions in the nervous system“

G. Publications

Original Papers (Peer Reviewed)

Rhein V, **Giese M**, Baysang G, Meier F, Rao S, Schulz KL, Hamburger M, Eckert A. *Ginkgo biloba extract ameliorates oxidative phosphorylation performance and rescues Aβ-induced failure.* PLoS One. 2010; 5(8): e12359.

Lim Y-A, Grimm A, **Giese M,** Mensah-Nyagan AG, Villafranca JE, Ittner LM, Eckert A, Gotz J. *Inhibition of the mitochondrial enzyme ABAD restores the amyloid-β-mediated deregulation of estradiol.* PLoS One. 2011; 6(12): e28887.

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Giese M, Unternährer E, Hüttig H, Beck J, Brand S, Calabrese P, Holsboer-Trachsler E, Eckert A. *BDNF – an indicator of insomnia?* Mol Psychiatry. 2013; 1-2.

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Giese M, Beck J, Brand S, Muheim F, Hatzinger M, Holsboer-Trachsler E, Eckert A. *A diurnal profile of serum BDNF before treatment is associated with therapy response after partial sleep deprivation in major depression.* J Psychiatr Res.

Abstracts

Giese M, Beck J, Brand S, Muheim F, Hatzinger M, Holsboer-Trachsler E and Eckert A. *Serum brain-derived neurotrophic factor levels as predictor of antidepressant therapy response.* ECNP Workshop on Neuropsychopharmacology for Young Scientists in Europe, Nice, France, 4-7 March 2010. Session: Molecular Neuropsychopharmacology. Poster n°012. Eur Neuropsychopharmacol. 2010 ; **20**: Supplement 1, S12-P.1.012.

Giese M, Beck J, Brand S, Muheim F, Hatzinger M, Holsboer-Trachsler E and Eckert A. *Serum brain-derived neurotrophic factor levels as predictor of antidepressant therapy response.* 30th SSBP Annual Meeting, Zurich, Switzerland, 18th March, 2010; Poster n°07.

Giese M, Lamontagne B, Krucker I, Meier F, Baysang G and Eckert A. *Beneficial effects of PUFA and fishoil on metabolic activity of neuronal cells.* 9th Conference of ISSFAL 2010, 29 May-2 June, 2010; Session: Lipids and Health, Maastricht, Netherlands. Poster n°P136, abstract book p.143, n°237.

Beck J, **Giese M**, Brand S, Muheim F, Hatzinger M, Eckert A, Holsboer-Trachsler E. *Sleep deprivation: therapeutic and research tool in depression.* 3rd Meeting of West European Societies of Biological Psychiatry: personalised medicine in Psychiatry: From dreams to reality. June 2-4, 2010; Berlin, Germany. Eur Arch Psychiatry Clin Neurosci 260 (Suppl 1):S14, Abstract 138.

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Giese M, Beck J, Brand S, Muheim F, Hatzinger M, Holsboer-Trachsler E and Eckert A. *Increased serumBDNF correlate with therapy response and improvement in psychological functioning in patients with major depressive episode.* SSN Annual Meeting 2011, Basel, Switzerland, March 26th, 2011; Poster n°K8.

Giese M, Beck J, Brand S, Muheim F, Hatzinger M, Holsboer-Trachsler E and Eckert A. *Serum brain-derived neurotrophic factor levels as predictor of antidepressant therapy response.* 24th ECNP congress, Paris, France, 3-7 September 2011; Session: Neuropharmacology. Poster n°P.1.c.018. Eur Neuropsychopharmacol. 2011 ; 20: Supplement 1, S12-P.1.012.

Giese M, Beck J, Brand S, Muheim F, Hatzinger M, Holsboer-Trachsler E and Eckert A. *Increase of serum BDNF level in depressive patients identifies therapy response and correlates with mood improvent.* 27. Symposium der AGNP, Munich, Germany, 5-8 October 2011.Poster n°29. Pharmacopsychiatry, 2011; 21-A37.

Giese M, Beck J, Brand S, Muheim F, Hatzinger M, Holsboer-Trachsler E, Eckert A. *Diurnal pattern of serum BDNF before partial sleep deprivation in stress-related mood disorders – an association with therapy response in major depression.* 41st annual ISPNE conference, New York, NY, 11-14 September 2012; European Journal of Psychotraumatology, 2012; Supplement 1 (3): 45-46.

Giese M, Beck J, Muheim F, Hatzinger M, Holsboer-Trachsler E, Eckert A. Presence of diurnal pattern of serum BDNF before partial sleep deprivation is associated with therapy response in major depression. 25th ECNP congress, Vienna, Austria, 13-17 October 2012. Session: Affective disorders

and antidepressants – Antidepressants (basic). Poster n°P.2.d.007. EurNeuropsychopharmacol. 2012; 22: Supplement 2, S271.

Eckert A, **Giese M**, Unternährer E, Hüttig H, Brand S, Calabrese P, Holsboer-Trachsler E. Decreased serum brain-derived neurotrophic factor (BDNF) levels in sleep-disturbed subjects. 25th ECNP congress, Vienna, Austria, 13-17 October 2012. Session: Basic and clinical neuroscience – Other. Poster n°P.1.i.005. Eur Neuropsychopharmacol. 2012; 22: Supplement 2, S271

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Book Chapter

Klinische Biomarkerforschung in der forensischen Psychiatrie. Römer KD, Hohmann M, **Giese M**, Graf M, Dittmann V und Eckert A. EFPPP Jahrbuch 2012: Empirische Forschung in der forensischen Psychiatrie, Psychologie und Psychotherapie. Jürgen L. Müller, Michael Rösler, Peer Briken, Peter Fromberger, Kirsten Jordan (Hrsg.). Medizinisch Wissenschaftliche Verlagsgesellschaft, Berlin. ISBN 978-3-941468-76-4. Kapitel I.6 Neurobiologische und neuropsychologische Aspekte. 48-57.

Travel Fellowships / Honours

Selection by the ECNP Committee for the ECNP Workshop on Neuropsychopharmacology for young Scientists in Europe, March 4th to 7th, 2010 in Nice, France.

Selection by the ECNP Committee to become an associate member of the European College of Neuropsychopharmacology (ECNP) for a period of 4 years in 2010.

SSBP 2010 poster award (2nd rank), 30th SSBP Annual Meeting, Zurich, Switzerland, 18th March, 2010.

SSN membership from 2011-2012.

ECNP 212 Poster/Travel Award, 25th ECNP Meeting, Vienna, Austria, October 13th -17th 2012.

Invited / Selected Oral Presentations

SSBP 30th Annual Meeting in Zurich, Switzerland, March 18th, 2010.
Poster Prize awardee, Session: Scientific session-oral presentation.

Young Researchers Meeting (Alzheimer forum) in Interlaken, Switzerland, November 19th, 2010. BDNF as marker for synaptic plasticity.

Neurex Workshop in Basel: Translational Approaches in Stress and Neurodegeneration, Switzerland, May 9th 2011. Young Investigator Session: Serum BDNF as predictor of antidepressant therapy response.

MitoNET Kongress – Mitochondriale Medizin 2012 in Bern, Switzerland, July 12-13, 2012. Session 2: Von der Klinik zu Pathomechanismen. Mitochondriale Dysfunktion bei Schizophrenie.

41st annual International Society of Psychoneuroendocrinology (ISPNE) conference, New York, NY, 11-14 September 2012. Effects of Traumatic Stress – Molecular and Hormonal Mechanisms. Free Communications: Diurnal pattern of serum BDNF before partial sleep deprivation in stress-related mood disorders – an association with therapy response in major depression