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

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SUMMARY AND CONCLUSIONS

The role of BDNF in Depression

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

BACKGROUND

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

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

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

RESULTS

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

PART I: Determinants

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

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

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

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

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

PART II: the neurotrophin hypothesis of depression

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

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

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

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

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

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

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

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

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

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

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

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

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

METHODS

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

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

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

DISCUSSION

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

Part I: determinants

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

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

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

Part I: the neurotrophin hypothesis

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

Serum BDNF concentrations - confirmative findings

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

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

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

Serum BDNF concentrations - non-confirmative findings

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

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

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

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

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

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

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

The neurotrophin hypothesis: BDNF val^{66}met

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

BDNF val^{66}met - confirmative findings

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

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

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

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

Conclusion

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

Future work – the drawing table

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