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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CHAPTER 6

Gender specific associations of serum BDNF concentrations in anxiety

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

ABSTRACT

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

INTRODUCTION

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

METHODS

Study population

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

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

BDNF protein measurements

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

Clinical characteristics

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

Covariates

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

Statistical analysis

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

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

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

RESULTS

Sociodemographic and clinical characteristics

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

Serum BDNF concentrations in patients and controls

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

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

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

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

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

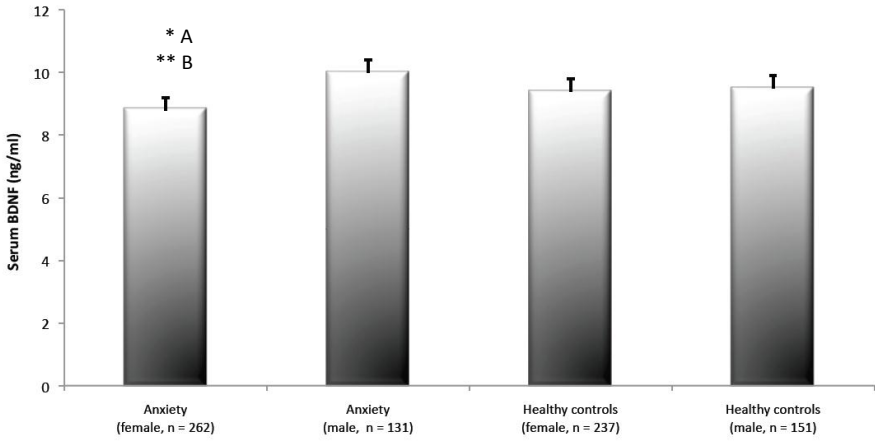

Figure 1. Plotted are mean serum BDNF concentrations by diagnosis and gender. Error bars reflect the *SEM*.
Serum BDNF concentrations are low in female patients with an anxiety disorder as compared with female controls (A: $d = 0.19$) and male patients with an anxiety disorder (B: $d = 0.30$).
The diagnosis-gender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

* Denotes statistical significance at $P < 0.05$.

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

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

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

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

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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