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 7

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

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

ABSTRACT

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

INTRODUCTION

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

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

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

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

METHOD

Patients and sample collection

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

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

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

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

Genotyping

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

BDNF measurements

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

Statistical analysis

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

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

RESULTS

Demographics

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

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

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

Abbreviations: BMI: Body Mass Index

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

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

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

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

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

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

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

Dose–response associations between childhood abuse and BDNF

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

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

Depression without comorbid anxiety

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

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

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

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

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

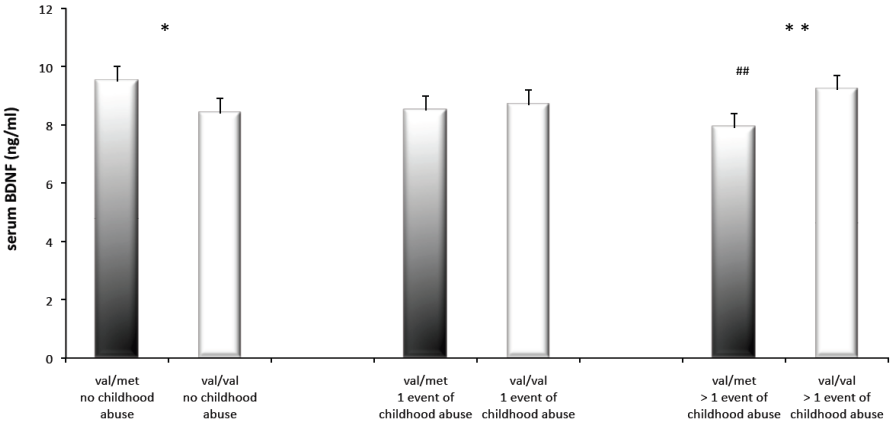

Figure 1. Mean serum BDNF concentrations by exposure to the number of childhood abuse types and val^{66}met. Error bars reflect the *SEM*. * denotes statistical significance at $P < .05$; ** denotes statistical significance at $P < .01$; ## denotes statistical significance at $P < .01$ when comparing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

Depression with comorbid anxiety

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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