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

Molendijk, M.L.

## CHAPTER 8

# BDNF val$^{66}$met affects hippocampal volume and emotion-related hippocampal memory activity

**Molendijk ML**
van Tol M-J
Spinhoven P
Aleman A
Penninx BWJH
Veltman D
Elzinga BM

Molendijk ML and van Tol M-J contributed equally

*Published as:* BDNF val$^{66}$met affects hippocampal volume and emotion-related hippocampal memory activity
*Translational Psychiatry* 2012; doi: 10.1038/ tp.2011.72

**SIGNIFICANCE:** In this study we further the understanding of the association of BDNF val$^{66}$met with hippocampal volume/functioning and cognitive performance. Critically, we take trauma/stress exposure into account. We find a small effect of val$^{66}$met on hippocampal volume and that trauma exposure in childhood accounts for individual differences in hippocampal encoding activity. This latter effect seems to 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 again, no effects no behavioral effects were observed alongside the neurobiological differences.

**ABSTRACT**

The val$^{66}$met polymorphism on the BDNF gene has been reported to explain individual differences in hippocampal volume and memory related activity. These findings, however, have not been replicated consistently and no studies to date controlled for the potentially confounding impact of early life stress, such as childhood abuse, and psychiatric status. Using structural and functional MRI we therefore investigated in 126 depressed and/or anxious patients and 31 healthy control subjects the effects of val$^{66}$met on hippocampal volume and encoding activity of emotional laden and neutral words, while taking into account childhood abuse and psychiatric status. Our results show slightly lower hippocampal volumes in carriers of a met allele ($n$ = 54) relative to val/val homozygotes ($n$ = 103; $P$ = 0.02, Cohen's $d$ = 0.37), which appeared to be independent of childhood abuse and psychiatric status. For hippocampal encoding activity we found a val$^{66}$met–word valence interaction ($P$ = 0.02) such that carriers of a met allele showed increased levels of activity in response to negative words. This, however, was only evident in the absence of childhood abuse, as abused val/val homozygotes showed hippocampal encoding activity for negative words that was comparable to that of carriers of a met allele. Neither psychiatric status nor memory accuracy did account for these associations. In conclusion, BDNF val$^{66}$met appears to have a small, yet significant, impact on hippocampal volume independently of childhood abuse and psychiatric status. Furthermore, early adverse experiences such as childhood abuse account for individual differences in hippocampal encoding activity of negative stimuli but this effect apparently manifests differently as a function of val$^{66}$met.

## INTRODUCTION

Brain-Derived Neurotrophic Factor (BDNF) regulates the sprouting of axons and dendrites in the hippocampus, a key structure for emotion and memory processing (Murer *et al*., 2001; Komulainen *et al*., 2008). Rodent studies, for example, have shown that BDNF modulates hippocampal neuronal differentiation (Taliaz *et al*., 2010) and hippocampal dependent memory (Choi *et al*., 2010). Moreover, human studies have reported a positive relation between BDNF concentrations, hippocampal volume, and memory performance (Gunstad *et al*., 2008; Erickson *et al*., 2010).

Studies focusing on a single nucleotide site in the DNA sequence of the BDNF gene; val$^{66}$met (a valine [val] to methionine [met] insertion at codon 66) have partly confirmed the associations of BDNF protein expression with neurobiological and behavioral abnormalities. Egan and colleagues (2003) showed *in vitro* that the met allele is linked to a reduced activity-dependent expression of BDNF in hippocampal neurons of rats, a finding that was replicated by Chen *et al.* (2004). In addition, studies have shown that in the hippocampus the met allele is associated with diminished levels of N-acetyl-aspartate, a putative marker for neuronal integrity (Stern *et al*., 2008). In line with these findings, some studies have shown that the met allele is associated with impaired episodic memory (Egan *et al*., 2003) and executive functioning (Rybakowski *et al*., 2003; Erickson *et al*., 2008). Structural and functional Magnetic Resonance Imaging (MRI) studies further suggest that carriers of a met allele have smaller hippocampal volumes relative to val/val homozygotes (Pezawas *et al*., 2004; Frodl *et al*., 2007) and altered hippocampal activity during the encoding of stimuli (Egan *et al*., 2003).

Nevertheless, these findings have not been consistently replicated (Schofield *et al*., 2009; Benjamin *et al*., 2010; Lau *et al*., 2010; Gerritsen *et al*., 2012) which might be due to the inclusion of small samples and task characteristics such as the emotional valence of the stimuli. Furthermore, the occurrence of early trauma such as childhood abuse and psychiatric status represent sources of variation in hippocampal volume and function (reviewed in Bremner *et al*., 2007; MacQueen and Frodl, 2011) that have not been taken into account in previous studies. In addition, gene-environment interactions have been reported between BDNF val$^{66}$met and abuse on brain structure and activity (Gatt *et al*, 2009; Juhasz *et al*., 2010). As a consequence, the earlier reported associations between BDNF val$^{66}$met and hippocampal structure and function might be (partly) dependent on a history of childhood abuse or on psychiatric status.

The goal of this study, then, was to evaluate the effects of val$^{66}$met on hippocampal volume and on encoding related hippocampal activity while taking into account the potential influence of childhood abuse and diagnostic status. Given earlier conflicting findings we further aimed to extend previous findings by examining the effects of neutral, positive, and negative emotional stimuli on hippocampal activity.

## MATERIAL AND METHODS

### *Subjects*

The data analyzed are from the imaging sample of the Netherlands Study of Depression and Anxiety (NESDA; Penninx *et al*., 2008; van Tol *et al*., 2010). Included in the sample were 301 subjects of whom 233 were patients with a current depressive and/or anxiety disorder and 68 healthy control subjects. Genetic and high-quality functional and structural MRI data were available for 157 persons of whom 126 were depressed and/or anxious patients and 31 were healthy controls. Subjects in the current study did not differ from subjects in the NESDA imaging sample ($N$ = 301) with regard to age ($P$ = .98), gender ($P$ = .22), and current diagnosis ($P$ = .07).

Subjects underwent imaging at three different locations in the Netherlands: Academic Medical Center (AMC), University of Amsterdam, University Medical Center Groningen (UMCG), and Leiden University Medical Center (LUMC). To be eligible subjects had to be between 18 to 57 years of age and fluent in Dutch.

Exclusion criteria were having an Axis-I disorder other than a depressive and/or anxiety disorder (Diagnostic and Statistical Manual of Mental disorders fourth edition (DSM-IV; APA 1994), being on antidepressant treatment other than SSRIs at a stable dose (WHO 2008), a history of a major internal or neurological disorder, dependency on alcohol and/or drugs, smoking > 5 cigarettes a day, or hypertension (> 180/130 mmHG). The protocol and procedures were approved by each of the Ethical Committees of participating institutes and all subjects signed an informed consent.

Diagnoses of depressive and anxiety disorders were established according to the criteria set forth in the DSM-IV (APA, 1994) on the basis of responses to the Composite International Diagnostic Interview 2.1 (CIDI) lifetime version (WIttchen *et al*., 1991), a reliable and validated diagnostic tool (Wacker *et al*., 2006). The severity of depressive and anxiety symptoms was assessed with the Montgomery Åsberg Depression Rating Scale (MÅDRS; Montgomery and Åsberg; 1979) and the Beck Anxiety Inventory (BAI; Beck *et al*., 1988;) which both have been shown to have excellent psychometric characteristics (Davidson *et al*., 1986; Kabacoff *et al*., 1997).

Childhood abuse was assessed retrospectively using a semi-structured childhood trauma interview de Graaf *et al*., 2004 a, b). In this interview, participants were asked whether they had experienced emotional neglect or psychological abuse, physical abuse, and/or sexual abuse before the age of 16 years. After an affirmative answer, subjects were asked for details on the frequency of the events. Based on the sum and the frequency of abusive events an index (range 0-8) was calculated for each subject (for details see Wiersma *et al*., 2009).

*Genotyping*

For a detailed description of the procedures we refer to Boomsma *et al*. (2008). In sum, variation at the val$^{66}$met locus was extracted from whole-genome data using PLINK software version 1.07 (www.pngu.mgh.harvard.edu). In our sample, 103 subjects were val/val homozygotes (65.6%) and 54 subjects carried a met allele (34.4%). Two subjects (1.3%) with the met/met genotype were merged with heterozygous subjects into a group of met allele carriers. Genotype counts were 82 val$^{66}$val, 42 val$^{66}$met, and 2 met$^{66}$met in the patient group and 21 val$^{66}$val, 10 val$^{66}$met, and 0 met$^{66}$met in the healthy control group. Patient and healthy control samples did not differ with regard to genotype distribution ($P$ = .77). Allele frequencies were in Hardy-Weinberg equilibrium in the GAIN-MDD sample in which the genotyping was performed ($N$ = 3,530, $\chi^2_1$ = 0.62, $P$ = .43) and in the sub-sample on which we present data ($n$ = 157, $\chi^2_1$ = 2.66, $P$ = .10).

*Memory Paradigm*

In the scanner subjects performed a subject-paced, event-related encoding task, similar to the paradigm described by Daselaar *et al*. (2003) and known to reliably activate the hippocampus. The task is described in detail elsewhere (van Tol *et al*., 2011). Briefly, during the encoding phase of the task 120 words (40 of neutral valence, 40 of positive valence, and 40 of negative valence) were presented in pseudo-randomized order. Subjects were instructed to classify these words according to valence. After a 10-minute retention interval, subjects were asked to complete a word recognition task. Subjects were instructed to indicate whether they had seen the word or whether the word was new. Discriminant accuracy was calculated as the proportion correctly recognized words minus the proportion false alarms (van Tol *et al*., 2011).

*Image acquisition and data handling*

Image acquisition and data handling are detailed elsewhere (van Tol *et al*., 2011, 2012). In sum, imaging data were collected using Philips 3-Tesla MRI scanners (Best, The Netherlands) using SENSE-6 and 8 channel

head coils (AMC and UMCG/LUMC respectively). Echo-planar images were obtained using a T2*-weighted gradient echo sequence with repetition time 2300 ms, a 30 ms echo time (UMCG 28ms), a matrix size of 96 × 96 (UMCG 64 × 64), producing 35 axial slices of 3 mm thickness direction interleaved, 2.29 × 2.29 mm in-plain resolution (UMCG 3 × 3). Anatomical imaging included a sagittal 3-D gradient-echo T1-weighted sequence with a repetition time of 9 ms and a 3.5 ms echo time producing slices with a voxel size of 1 × 1 × 1 mm. Imaging data were preprocessed with SPM5 (Statistic Parametric Mapping, London, UK; www.fil.ion.ucl.ac.uk/).

Preprocessing of the data included reorientation of the functional images to the anterior commissure, slice time correction, image realignment, registration of the T1-scan to the mean image, warping to Montreal Neurological Institute (MNI) space as defined by the SPM5 T1-template, reslicing to 3 × 3 × 3mm voxels, and spatial smoothing using an 8 mm FWHM Gaussian kernel. Haemodynamic responses to each stimulus were modeled with a delta function convolved with a synthetic haemodynamic response function and modulated using response times.

Contrast images for *subsequent hits* versus *baseline* were calculated for the neutral, positive, and negative word condition per subject on a voxel-by-voxel basis, based on subsequent recognition success and entered in a 2 (group: val/val homozygotes versus met carriers; independent factor) by 3 (condition: neutral, positive, negative (> baseline); dependent factor) Mancova with age, education and scan center as covariates. Mean BOLD signal change during successful encoding in the left and right hippocampus was extracted per condition (neutral/positive/neutral > baseline) using the MARSBAR toolbox (Brett *et al*., 2002). The hippocampal masks of the Automated Anatomical Labeling software package, implemented in the WFU Pick Atlas toolbox (Maldjian *et al*., 2003) were used to define the left and right hippocampal region.

Anatomical images were processed using an optimized Voxel Based Morphometry approach, following the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL; Ashburner, 2007) using SPM5 software implemented in Matlab 7.1.0 (The MathWorks Inc., Natick, MA, USA). For details see van Tol *et al*. (2011, 2012). To test for differences in regional brain volume, an independent samples *t*-test was set up for a voxel-wise comparison of the grey matter density images of the val/val homozygotes and met carriers, with age, scan center and total gray matter volume as covariates. Following a similar approach as for signal change extraction, the mean volume of the left and right hippocampus was additionally extracted. Data were exported to SPSS 18.0 (Chicago, IL, USA) for further analysis.

*Statistical analyses*

Computations were performed in SPSS 18.0. A *P* value of < .05 (2-tailed) was considered as the threshold for statistical significance. Demographical and clinical characteristics between groups were compared using Student's *t*-tests for continuous- and $\chi^2$-tests for categorical data.

Main effects of val$^{66}$met on right, left, and total hippocampal volume were calculated using a Repeated Measures (RM) Ancova with left versus right hippocampal volume as the within-subjects factor and age, gender, number of years of education, SSRI use, alcohol use, scan site, and total gray matter volume as covariates. Ancova's were used to assess the effects of val$^{66}$met on memory accuracy and hippocampal activity during the encoding of neutral, positive, and negative words. To address val$^{66}$met–valence interaction effects on memory accuracy and hippocampal encoding activity we ran RM Ancova's with word valence (positive versus neutral and negative versus neutral) as within-subject factor and age, gender, number of years of education, SSRI use, alcohol use, scan site, hippocampal volume, memory accuracy, and handedness as covariates. If indicated by between-group differences in memory accuracy, accuracy scores were included as covariates in the analyses on hippocampal encoding activity.

Possible interaction effects of val$^{66}$met with abuse and diagnosis (dummy variables coding for healthy, depressed, depressed-anxious, and anxious) on hippocampal volume, memory accuracy, and hippocampal encoding activity were evaluated using hierarchical stepwise regression analyses if indicated by statistically significant associations in the above described analyses. Regression analyses consisted of three steps: (**I**) covariates, (**II**) val$^{66}$met, childhood abuse, and diagnosis, and (**III**) the interaction terms val$^{66}$met × abuse and val$^{66}$met × diagnosis. Analyses were rerun with lifetime instead of current diagnosis. Tolerance of the predictors and normality of error variances were verified.

To assess regional specificity of val$^{66}$met within the hippocampus, voxel-wise analyses were repeated on the gray matter density maps and contrast maps reflecting encoding related activity using SPM5, with the threshold set at $P < .001$, uncorrected. For regions outside the hippocampus, a threshold of $P < .05$, FWE corrected was set.

**RESULTS**

The overall sample ($N = 157$) had a mean age of 37.39 ± 10.08 years and included 100 women (63.7%). Demographical and clinical characteristics of the sample are given in **Table 1 ↓** by BDNF genotype. There were no statistically significant differences between the genotype groups in terms of demographical and clinical variables. Furthermore, val$^{66}$met was not differentially associated with exposure to childhood abuse (dichotomous nor with exposure to the specific types of childhood abuse (all *P*-values > .75).

**Table 1**. Demographic and clinical characteristics (mean ± *STD* or percentages) by BDNF genotype

| | | val$^{66}$val ($n$ = 103) | val$^{66}$met ($n$ = 54) | *P*-value |
|---|---|---|---|---|
| Females | % | 64.1 | 63.0 | = .89 |
| Age | | 37.1 ± 10.0 | 37.9 ± 10.4 | = .65 |
| Education (years) | | 12.4 ± 3.0 | 12.6 ± 3.3 | = .70 |
| Smoker | % | 33.0 | 23.2 | = .14 |
| Alcohol use | % | 56.2 | 60.0 | = .58 |
| SSRI use | % | 30.1 | 20.4 | = .19 |
| Right handed | % | 91.3 | 94.4 | = .48 |
| Childhood trauma index range 0 -8) | | 1.6 ± 2.0 | 1.7 ± 2.3 | = .87 |
| Diagnostic status | | | | = .78 [1] |
| Healthy controls | % | 20.4 | 18.5 | = .78 |
| Depression | % | 22.3 | 25.9 | = .61 |
| Anxiety [2] | % | 20.4 | 14.8 | = .39 |
| Depression and anxiety [2] | % | 20.4 | 24.1 | = .59 |
| Depression severity, MÅDRS | | 11.6 ± 8.8 | 13.6 ± 11.6 | = .23 |
| Anxiety severity, BAI | | 11.7 ± 9.1 | 13.3 ± 11.2 | = .84 |

*Abbreviations:* BAI, Becks Anxiety Inventory; MADRS, Montgomery Åsberg Depression Rating Scale; SSRI, Selective Serotonin Reuptake Inhibitor
[1] Chi-square test (3 *df*) for differences in distribution of the val and the met allele over diagnoses
[2] Included a diagnosis of social phobia, panic disorder, generalized anxiety disorder, and/or agoraphobia

*BDNF val$^{66}$met and hippocampal volume*

Total hippocampal volume was smaller in carriers of a met allele relative to val/val homozygotes ($F_{1,180} = 5.33$, $P = .02$; standardized Cohen's $d = 0.38$; see **Figure 1 ↓** and **Table 2 ↓** for covariate adjusted means on total, right, and left hippocampal volume ± Standard Error [*SE*]). No interaction of val$^{66}$met × right versus

left hippocampus was observed ($P$ = .63). BDNF val$^{66}$met had no effect on total gray matter volume ($P$ = .60). Voxelwise analyses of the hippocampus confirmed these findings, with the peak voxel located in the posterior part of the hippocampus (MNI coordinate: Right hippocampus: [x = 18 y = -33 z = 8 and x = 21 y = -30 z = -4], Z = 3.61/3.42, k = 29/17, $P_{FWE\text{-}ROI}$ = .018; Left hippocampus: [x = -18 y = -36 z = 8], $Z$ = 3.17, k = 4, $P_{FWE\text{-}ROI}$ = .062).

Regression analyses were used to evaluate whether the smaller hippocampal volume in met carriers as compared to val/val homozygotes were moderated by the effects of abuse or diagnostic status. Main effects of childhood abuse and psychiatric status, and interaction effects of val$^{66}$met with childhood abuse and psychiatric status on hippocampal volume were not observed (all $P$'s > .10). The main effect of val$^{66}$met remained statistically significant after the inclusion of childhood abuse and psychiatric status in the model ($B$ = -0.13, 95% Confidence Interval [$CI$] = -0.24 to -0.02, $P$ = .02). Similar results were obtained in analyses with lifetime instead of current diagnosis and in analysis in which continuous measures for childhood abuse and depression severity were included as predictors (data not shown). No effect of BDNF val$^{66}$met was observed on other structures at the set threshold (data not shown).

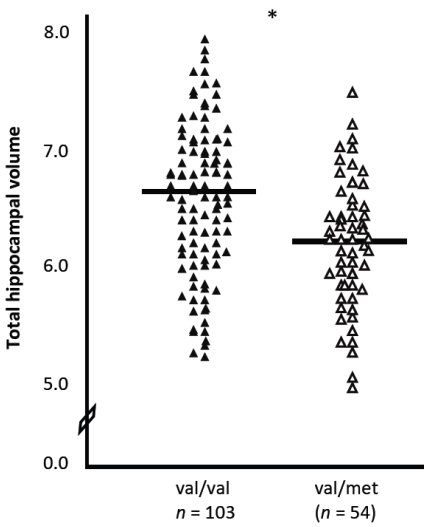


**Figure 1.** Hippocampal volume by val/val and val/met genotype. Data are covariate adjusted (see the method section).

* Denotes statistical significance at $P$ < 0.05 ($d$ = 0.38).

**Table 2** Cerebral and hippocampal volumes and hippocampal related encoding activity (mean ± *SEM*) by BDNF genotype and word valence (neutral, positive, and negative)

| | val$^{66}$val ($n$ = 103) | val$^{66}$met ($n$ = 54) | $P$ value |
|---|---|---|---|
| Total grey matter volume [1] | 736.57 ± 5.35 | 731.66 ± 7.45 | = .60 |
| Hippocampal volume [1, 2] | | | |
| Total | 6.45 ± 0.03 | 6.31 ± 0.04 | = .02 |
| Right | 3.06 ± 0.02 | 2.99 ± 0.02 | = .01 |
| Left | 3.39 ± 0.02 | 3.31 ± 0.02 | = .05 |
| Hippocampal encoding activity [1, 3] | | | |
| Neutral words | 0.15 ± 0.04 | 0.16 ± 0.06 | = .97 |
| Positive words | 0.15 ± 0.05 | 0.23 ± 0.07 | = .30 |
| Negative words | 0.20 ± 0.05 | 0.36 ± 0.06 | = .04 |

[1] All mean values are corrected for gender, age, years of education, SSRI and alcohol use, and site of scanning
[2] Mean values are additionally corrected for total cerebral grey matter volume
[3] Mean values are additionally corrected for total hippocampal volume

*BDNF val$^{66}$met and task performance*

Persons who were val/val homozygotes did not differ from met carriers with regard to the discriminant accuracy of neutral, positive, and negative words (all *P's* > .35). There also were no overall differences in discriminant accuracy as a function of genotype (covariate adjusted means ± SE: val/val homozygotes = 0.57 ± 0.01 versus met carriers = 0.58 ± 0.02; *P* = .85). Interaction effects of val$^{66}$met and word valence on memory accuracy were not observed (all *P*'s > .10). Furthermore, memory accuracy was unrelated to hippocampal volume (Pearson's *r* = 0.13; *P* = .10) and to hippocampal encoding activity (*r* = 0.04; *P* = .66).

*BDNF val$^{66}$met and hippocampal activity*

Main effects of val$^{66}$met and word valence on hippocampal activity during the encoding of neutral and positive words were not observed (see **Table 2 ↑**). However, val$^{66}$met interacted with neutral versus negative word valence (*P* = .02) such that hippocampal activity was higher in carriers of a met allele in the negative word condition relative to hippocampal activity in the neutral word condition (Bonferroni corrected *P* = not significant). This was not observed in val/val homozygotes (see **Figure 2 ↓** and **Table 2 ↑** for covariate adjusted means ± *SE* by word valence). No val$^{66}$met-neutral versus positive word valence interaction effect on encoding activity was found (*P* = .17). Effects of lateralization were not observed. Voxel-wise analyses located the peak voxel of the interaction between negative versus neutral encoding times val$^{66}$met cluster at the left posterior hippocampus ([x = -21 y = -27 z = -6], $F_{1, 461}$ = 14,11, *Z* = 3.55, $P_{FWE\_ROI}$ = .024, K [number of voxels] = 15).

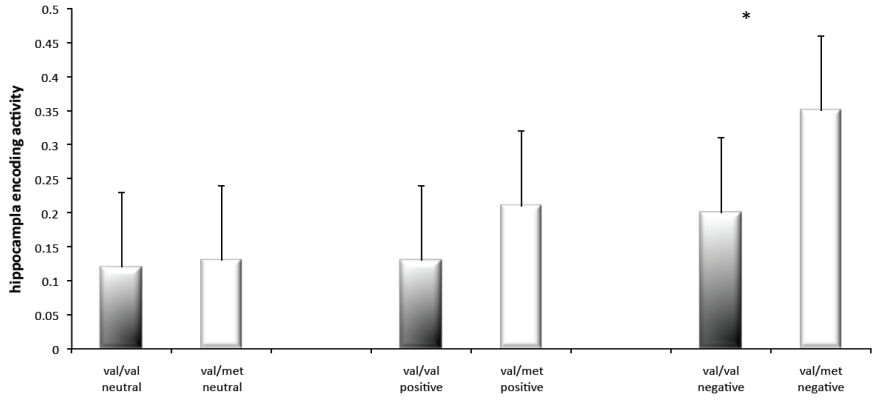


**Figure 2.** Plotted are covariate adjusted mean total hippocampal activity levels during encoding by stimulus valence and val$^{66}$met genotype (val/val *n* = 103; val/met *n* = 54). Error bars reflect the *SEM*.

* denotes statistical significance

Exploratory voxel-wise whole brain analyses showed no statistical significant effects of val$^{66}$met and val$^{66}$met-word valence interactions in brain areas other than the hippocampus at the a priori set threshold of *P* < .05, FWE corrected.

Regression analyses were used to evaluate whether the higher hippocampal activity during the encoding of negative words were moderated by the effects of abuse or diagnostic status. Hippocampal encoding activity in response to words of negative valence was higher in abused subjects as compared to non-abused subjects (*B* = 0.16, 95% CI = 0.05 to 0.28, *P* = .007). In addition we found a val$^{66}$met-childhood abuse interaction effect (*B* = -0.10, 95% CI = -0.17 to -0.02, *P* = .01) showing that childhood abuse predicted increased hippocampal activation in response to negative words in val/val homozygotes (*P* = .009) but not in carriers of a met allele (*P* = .34) (see **Figure 3 ↓**). Effects of psychiatric status (lifetime and current) and val$^{66}$met by psychiatric status interaction effects were not observed (all *P*'s > .10). Adding memory accuracy as a predictor to the model did not change our results (data not shown) making it unlikely that these results are accounted for by genotype differences regarding attention or effort.

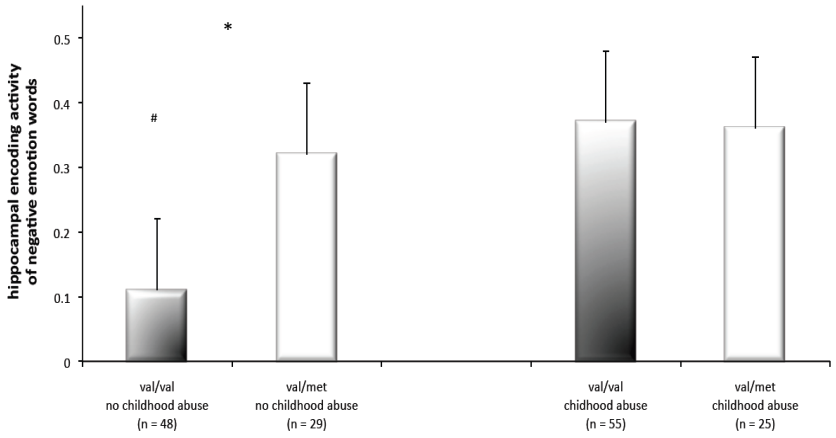


**Figure 3.** Plotted are covariate adjusted mean total hippocampal activity levels during the encoding of negative words by childhood abuse exposure and val$^{66}$met.
Error bars reflect the *SEM*.
The val$^{66}$met – childhood abuse interaction effect is statistically significant at $P$ = .01.

* denotes statistical significance at $P$ < .05. # denotes statistical significance at $P$ < .05 for the difference between abused and non-abused val homozygotes

## DISCUSSION

We addressed the effects of val$^{66}$met on hippocampal volume and function while taking into account the possible confounding effects of childhood abuse and psychiatric status.

In line with some previous studies (Szeszko *et al*., 2005; Bueller *et al*., 2006; Frodl *et al*., 2007) but not all (*e.g.,* Gerritsen *et al*., 2012) we find smaller hippocampal volumes in carriers of a met allele relative to val/val homozygotes. This effect has generally been explained by abnormal intracellular trafficking and impaired activity secretion of BDNF, and by extension aberrant (trophic support in carriers of a met allele relative to the val/val homozygotes that have been shown in *in vitro* experiments (Egan *et al*., 2003; Chen *et al*., 2004). But since atrophy of the hippocampus has also been associated with (early) stress and/or current or remitted depression (MacQueen and Frodl, 2011) it is crucial to exclude the possible confounding effects of these variables. Our data suggests that the association between the met allele and hippocampal volume is independent of childhood abuse. This finding is at odds with those of Gatt and colleagues (2009) who modeled the interaction of early life stress and val$^{66}$met in the prediction of hippocampal volume and found that the combination of carrying a met allele and being exposed to early life stress was associated with smaller hippocampal volumes in healthy adults. It could be that the observed discrepancy between the results of Gatt *et al*. (2009) and ours might be explained by a broader definition of early life stress by Gatt and colleagues (2009) who included for example also *illness and exposure to natural disasters* as stressful events whereas we focused on childhood abuse including physical, sexual, and emotional abuse. Furthermore, the study subjects of Gatt *et al*. (2009) were all healthy control subjects ($N$ = 89) whereas we studied mostly patients. However, exactly how these differences between the studies could have led to a different pattern of results is unclear. In line with Frodl and colleagues (2007), we show that lifetime and current psychiatric status does not thrive the val$^{66}$met genotype effect on hippocampal volume, providing evidence for a direct association between the met allele and small hippocampal volume that further appears to be specific to the hippocampus.

In addition to reduced hippocampal volume, we show that val$^{66}$met interacts with word valence such that encoding activity is increased in carriers of a met allele during the negative word condition and not in the neutral or positive word condition. This effect was not observed in other brain areas than the hippocampus and is consistent with some studies in which emotional stimuli were used (*e.g.,* Dennis *et al*., 2010). We could not replicate the finding of higher hippocampal activation in carriers of a met allele in response to neutral stimuli that was reported in the seminal study by Egan *et al*. (2003). On the basis of a recent study that showed that negative affectivity increased more in response to social stress in met carriers as compared to val/val homozygotes (Wichers *et al*., 2008) one may speculate that carriers of a met

allele are more sensitive or reactive to negative stimuli. Owing to a possible relation between higher hippocampal activity and psychopathology (Thomaes *et al*., 2009) this finding might concur with studies that show a link between the met allele and depression (reviewed in Verhagen *et al*., 2010).

We further found, in line with some studies that childhood abuse predicts higher levels of hippocampal encoding activity (Werner *et al*., 2009; Heim *et al*., 2010). However, from our data it appears that childhood abuse is associated with a relative increase in hippocampal activity in val/val homozygotes only and not in carriers of a met allele. Although speculative, an interpretation may be that higher levels of hippocampal activity after exposure to childhood abuse in val/val homozygotes reflect a higher sensitivity for emotionally negative stimuli in that in carriers of a met allele is present regardless of exposure to childhood abuse. This idea is in line with studies that report hippocampal dysfunction in various severe psychiatric illnesses, particularly if exposure to childhood abuse is documented (Thomaes *et al*., 2009; Heim *et al*., 2010; MacQueen and Frodl, 2011).

Despite differences in hippocampal volume and activity between val/val homozygotes and carriers of a met allele we did not find differences in memory accuracy and clinical variables (e.g., depression severity) as a function of BDNF genotype. This may suggest that our findings are relevant for both healthy individuals and patients and also is pertinent to the debate on the relationship between hippocampal volume and function with behavioral performance. However, with regard to the absence of associations between hippocampal volume, hippocampal function, and memory performance, a recent review on 80 studies showed, in line with our findings, that the model: *a bigger brain structure → greater brain response → better performance* may not reflect reality (Eyler *et al*., 2010).

A notable strength of our study is that the findings are derived from a genetically homogeneous sample making it unlikely that our results are devoid by population stratification (Cardon and Palmer, 2003). Furthermore, we studied the effects of val$^{66}$met in the context of childhood abuse and emotional valence of stimuli, and our results clearly highlight the importance of including such variables. A few weaknesses of our study also merit attention. Obviously, we cannot exclude the possibility that other polymorphisms on the BDNF gene or on other genes, notably those that constitute the neurotrophic pathway (e.g., CREB1 and NTRK2; see for example Juhasz *et al*., 2010) might have contributed to the effects that we observed. With regard to our self-reported measurement of childhood abuse it should be noted that the validity and reliability of recall might vary by diagnosis and time since abuse took place. Furthermore, in the face of negative findings statistical power is important to take into account. Overall we had a comparatively large sample size, but our analysis on psychiatric status might have been underpowered because patient samples may have been too small (*e.g.,* only 31 healthy control subjects) to detect main effect sizes that are reported to be moderate at best (Campbell *et al*., 2004; Videbech *et al*., 2004). Finally, although we speculate that carriers of a met allele are more reactive to emotionally negative laden stimuli as compared to val/val homozygotes we are not able to confirm this because we have no subjective ratings of the stimuli by our participants.

In sum, our results suggest that BDNF val$^{66}$met has a small effect on hippocampal volume and this effect appears to be independent of childhood abuse and psychiatric status. Furthermore, gene-environment interactions between val$^{66}$met and childhood abuse account for individual differences in hippocampal encoding activity of negative stimuli. Important venues for future research are to delineate the exact mechanisms, *in vivo*, through which the met allele produces its effect on hippocampal volume and function. In addition, it remains to be investigated, in longitudinal designs, whether or not the effects of val$^{66}$met on hippocampal volume and activity are predictive for individual cognitive functioning and psychological wellbeing.