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RESEARCH ARTICLE

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The role of *BDNF* methylation and Val^{66}Met in amygdala reactivity during emotion processing

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Abstract

Epigenetic alterations of the brain-derived neurotrophic factor (*BDNF*) gene have been associated with psychiatric disorders in humans and with differences in amygdala *BDNF* mRNA levels in rodents. This human study aimed to investigate the relationship between the functional *BDNF*-Val^{66}Met polymorphism, its surrounding DNA methylation in *BDNF* exon IX, amygdala reactivity to emotional faces, and personality traits. Healthy controls (HC, n = 189) underwent functional MRI during an emotional face-matching task. Harm avoidance, novelty seeking and reward dependence were measured using the Tridimensional Personality Questionnaire (TPQ). Individual *BDNF* methylation profiles were ascertained and associated with several BDNF single nucleotide polymorphisms surrounding the *BDNF*-Val^{66}Met, amygdala reactivity, novelty seeking and harm avoidance. Higher *BDNF* methylation was associated with higher amygdala reactivity (x = 34, y = 0, z = −26, $t_{(166)}$ = 3.00, TFCE = 42.39, $p_{(FWE)}$ = .045), whereby the *BDNF*-Val^{66}Met genotype per se did not show any significant association with brain function. Furthermore, novelty seeking was negatively associated with *BDNF* methylation (r = −.19, p = .015) and amygdala reactivity (r = −.17, p = .028), while harm avoidance showed a trend for a positive association with *BDNF*

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methylation (r = .14, p = .066). The study provides first insights into the relationship among *BDNF* methylation, *BDNF* genotype, amygdala reactivity and personality traits in humans, highlighting the multidimensional relations among genetics, epigenetics, and neuronal functions. The present study suggests a possible involvement of epigenetic *BDNF* modifications in psychiatric disorders and related brain functions, whereby high *BDNF* methylation might reduce *BDNF* mRNA expression and upregulate amygdala reactivity.

KEYWORDS
amygdala reactivity, BDNF, fMRI, methylation, novelty seeking, Val^{66}Met

1 | INTRODUCTION

The brain derived neurotrophic factor (BDNF) has been shown to play a crucial role in neural development, function and plasticity of the amygdala, mediating anxiety-like behaviors (Sagarkar et al., 2017). A wide range of studies have linked BDNF expression to the etiology and pathophysiology of several psychiatric disorders associated with deficient amygdala function, including mood disorders, anxiety disorders (Gottschalk & Domschke, 2017; Ikegame et al., 2013), eating disorders, and personality disorders (Thaler et al., 2014). Based on its broad neuronal impact, the genetic and epigenetic regulation of BDNF became a main topic in the field of molecular psychiatry. Various studies focused on a frequent single-nucleotide polymorphism (SNP) in the corresponding gene *BDNF* at nucleotide 196 (G/A; rs6265), which produces an amino acid substitution (valine to methionine) at codon 66 (Val^{66}Met) in the proregion of the BDNF protein. The Met allele of this SNP has been shown to decrease activity-dependent intracellular trafficking and secretion of neuronal BDNF (Chen et al., 2004; Egan et al., 2003), leading to increased anxiety-related behaviors (Chen et al., 2006). In addition, studies revealed associations of the Val^{66}Met SNP with amygdala reactivity (Lau et al., 2010; Montag, Reuter, Newport, Elger, & Weber, 2008), with a deficit in amygdala habituation, particularly for emotional pictures (Perez-Rodriguez et al., 2017), and anxiety-related endophenotypes, such as harm avoidance (Jiang et al., 2005; Montag et al., 2010b). However, the direction of Val^{66}Met SNP impact on anxiety-related endophenotypes is still unclear, for instance controls carrying a ^{66}Met allele show lower neuroticism scores but also a trend for higher harm avoidance scores (Frustaci, Pozzi, Gianfagna, Manzoli, & Boccia, 2008). Taken together, although associations of the Val^{66}Met polymorphism with anxiety traits were reported, the results remain inconclusive (Frustaci et al., 2008), suggesting further underlying variables and biological mechanisms involved in the regulation of the BDNF system.

Recently, the role of epigenetics, and in particular of DNA methylation, gained focus as one such mechanism. In an enrichment microarray analysis of the methylation status of several genetic *BDNF* regions, Mill et al. (2008) could identify an association of the Val^{66}Met polymorphism and the methylation status of some of the surrounding CpG sites in exon IX. Carrying a guanine nucleotide (Val allele) resulted in an additional CpG site, which in turn lead to an increased methylation of surrounding CpG sites. Recent research revealed that DNA methylation of the *BDNF* gene broadly affects *BDNF* mRNA expression (Nagy, Vaillancourt, & Turecki, 2018), whereby high *BDNF* gene methylation goes along with low *BDNF* mRNA levels. Numerous studies have associated *BDNF* gene methylation with certain psychiatric disorders (anxiety disorder, major depression, bipolar disorder, borderline personality disorder, schizophrenia) (Zheleznyakova, Cao, & Schiöth, 2016) and personality traits like novelty seeking (Duclot & Kabbaj, 2013), highlighting the potential of *BDNF* methylation as a biomarker of psychiatric diseases. Focusing on amygdala functions, animal studies showed epigenetic modifications of *BDNF* to be associated with *BDNF* mRNA levels in the amygdala (Sagarkar et al., 2017). However, in humans, the associations of BDNF genotype, *BDNF* methylation, and amygdala reactivity have not been investigated yet.

Therefore, in the present study, we aimed to investigate the relationship between the *BDNF* Val^{66}Met polymorphism, *BDNF* DNA methylation, amygdala reactivity and psychological phenotype in a large sample of n = 189 healthy participants. Furthermore, we additionally investigated the potential—confounding—effects of BMI, since neurotrophic factors, particularly the BDNF, is associated with the control of body weight and mutations in BDNF encoding genes lead to insatiable appetite and severe obesity (Xu & Xie, 2016). First, based on previous research, we hypothesized that the *BDNF* methylation in exon IX is associated with the Val^{66}Met polymorphism. Second, we investigated possible main and interaction effects of *BDNF* methylation and the Val^{66}Met polymorphism on amygdala reactivity. We hypothesized that amygdala reactivity to negative emotional stimuli is associated with *BDNF* methylation and the Val^{66}Met polymorphism. Finally, the effects of *BDNF* methylation, the Val^{66}Met polymorphism and limbic brain reactivity on novelty seeking, harm avoidance as well as reward dependence for the sake of completeness were exploratively investigated, based on first evidence that have shown associations with BDNF in human and rodent studies (Duclot & Kabbaj, 2013; Jiang et al., 2005; Montag, Basten, Stelzel, Fiebach, & Reuter, 2010a; Montag, Markett, et al., 2010b).

2 | METHODS AND MATERIALS

2.1 | Subjects

In the present study n = 189 right-handed Caucasian healthy participants were analyzed. Participants responded to local newspaper ads and public notices. They were thoroughly investigated by experienced psychologists and free from any life-time history of psychiatric disorders according to DSM-IV criteria (American Psychiatric Association, 1994), as diagnosed with the SCID interview (Wittchen, Zaudig, & Fydrich, 1997). Exclusion criteria encompassed any neurological abnormalities, history of seizures, head trauma or unconsciousness, intake of any psychotropic medication, and the usual MRI-contraindications. Harm avoidance (HA), novelty seeking (NS), and reward dependence (RD) were measured using the Tridimensional Personality Questionnaire (TPQ) (Cloninger, Przybeck, & Svrakic, 1991). Table 1 lists sociodemographic and questionnaire data of study participants clear from fMRI movement effects (see below). The study was approved by the Ethics Committee of the University of Münster. After complete description of the study to the participants, written informed consent was obtained. Participants received a financial compensation.

2.2 | DNA analysis

Venous blood samples were taken from the 189 participants by default between 5 and 7 p.m., within 30 min postscanning. DNA was extracted as recommended by the manufacturer (FlexiGene DNA Kit; Qiagen, Germany) and dissolved in 25 mM Tris–HCL buffer (pH 7.8). Concentrations were ascertained by measurement of 260/280 nm absorbance (GENios Pro; Tecan, Germany), and DNAs diluted to 25 ng/μL.

DNA aliquots (500 ng) of participants were converted plus No Template Control with sodium bisulfite using EZ DNA Methylation Kit according to the manufacturer's instructions (Zymo Research, HiSS Diagnostics GmbH, Germany) with minor modifications: an incubation time of 5 min was included to step 12 after adding 10 μL M-Elution Buffer and prior to centrifugation; this modified step was repeated with 12 μL M-Elution Buffer (both eluates were pooled). For polymerase chain reaction (PCR) amplification of converted DNAs, Methyl Primer Express Software v1.0 and Primer Express Software v2.0 (Applied Biosystems by Thermo Fisher Scientific, Germany) were utilized for the design of bisulfite sequencing primers. These were chosen to encompass Val^{66}Met SNP rs6265 and its closest surrounding CpG sites (Figure S1), tested for specificity via the BiSearch web server (Arányi, Váradi, Simon, & Tusnády, 2006; Tusnády, Simon, Váradi, & Arányi, 2005), and extended by 5′ tails enriched of C-bases for optimal PCR performance (F: 5′-TCCCATTTTATATTTTGGTTGTATGAAGGTTG, R: 5′-GGGGAAAACACTTAACTACTAAACATCACCCT). For standard PCRs of bisulfite converted DNA aliquots (30 ng), 0.8 μM of each extended primer and 1X ZymoTaq PreMix (Zymo Research, HiSS Diagnostics GmbH, Germany) were used in 20 μL final volumes with following conditions: 95°C for 10 min, 40 cycles of 94.5°C for 1 min + 58°C for 1 min + 72°C for 2 min, followed by 72°C for 7 min. After PCR clean-up with Vacuum Manifold and MultiScreen HTS Filter Plates following the manufacturer's instructions (Millipore GmbH, Germany) sequencing reactions were performed with forward primer only using Big Dye Terminator chemistry (v3.1 Cycle Sequencing Kit, Applied Biosystems by Thermo Fisher Scientific, Germany). Ten microliters final volumes contained 3 μL cleaned PCR product and 7 μL Mastermix composed of 1 μL BigDye, 2 μL 5X Sequencing Buffer and 0.3 μM primer. Reaction conditions were 96°C for 1 min followed by 25 cycles of 96°C for 10 s + 50°C for 4 min. After removal of excess Dye Terminator using Sephadex-MultiScreen-HV plate system as recommended (Millipore GmbH, Germany), cleaned sequencing products were run on a 3,730 DNA analyser (Applied Biosystems by Thermo Fisher Scientific, Germany). Electropherograms were manually checked for genotypes of Val^{66}Met SNP rs6265, mean fluorescence intensities, overall quality, and analyzability of CpG sites within the sequenced amplicon using sequence scanner software v1.0 (Applied Biosystems by Thermo Fisher Scientific, Germany). Additionally, genotypes of additional variants spanning the *BDNF* gene around rs6265, altogether eight variants, were determined: rs1519480, rs6265, rs11030101, rs11030104, rs7127507, rs988748, rs962369, rs12273363. Linkage disequilibrium (LD) analysis revealed that tightly linked variants rs1519480 and rs12273363 should thus provide additional and helpful information and are most likely to contribute to the relationship between genotype and DNA methylation status of the *BDNF* gene (for details see Supplemental Material).

For uncovering the individual methylation profiles, quantitative analysis of CpG site specific methylation (relative peak heights C/C + T) was performed with Epigenetic Sequencing Methylation analysis software (ESME) as recommended (Lewin, Schmitt, Adorjan, Hildmann, & Piepenbrock, 2004) and described previously (Domschke et al., 2012; Schartner et al., 2017; Tadić et al., 2014; Ziegler et al., 2016; Ziegler et al., 2018). This included quality control, correction for incomplete bisulfite conversions, normalization of signals, and alignment of own generated sequence trace files and reference sequences (public databases). The central CpG site at position 172–173 includes the Val^{66}Met SNP rs6265 (at position 173) which allowed robust ESME data analysis only for participants homozygous for the frequent

TABLE 1 Sociodemographic, questionnaire, and genotype data of participants (n = 173)

	M ± *SD*/frequency
Age	35.2 ± 10.9
Sex (m/f)	82/91
BMI	23.7 ± 3.3
Education	15.4 ± 2.2
BDNF rs6265 (Val/Val, Val/met, met/met)	118/47/8
BDNF rs6265 (Val/Val, Val/met and met/met)	118/55
TPQ-HA	9.5 ± 4.4
TPQ-NS	17.7 ± 3.8
TPQ-RD	17.8 ± 4.0

Abbreviations: BMI, body mass index; HA, subscales harm avoidance; NS, novelty seeking; RD, reward dependence; TPQ, Tridimensional Personality Questionnaire.

G-allele (two CpG sites present), but not for heterozygous G/A participants (one CpG plus one CpA site) or homozygous A/A participants (two CpA sites). Therefore the methylation rates of this CpG site at position 172 bp were excluded for further analysis.

For quality control, standard PCRs and sequencing reactions were performed each in duplicate and checked for concordance (*SD* of mean methylation rate per participant to be ≤0.05) or extended to triplicate sequencing reactions, to include only mean data with *SD* ≤0.05 for further analysis (see below). Further, DNAs of seven random participants were independently three or four times sodium bisulfite converted followed by independent standard PCRs and sequencing reactions up to three times for each converted DNA. This resulted in at least quadruplicate (fourfold) sequence data sets up to twelvefold data sets (2 DNAs: 4 sets, 2 DNAs: 6 sets, 1 DNA: 8 sets, 2 DNAs: 12 sets) which revealed high concordance: *SD* of mean methylation rates within the same DNA <0.05 at all CpG sites. A boxplot depicting median and quartiles for all methylation CpG Sites can be found in Figure S2. All DNA analysis for quantitative investigation of individual methylation profiles and genotyping was performed analogous to previous research (Dannlowski et al., 2014) again by investigators blind for participant characteristics, structural or functional imaging data.

2.3 | fMRI methods

The experimental fMRI paradigm was frequently used to elicit a robust and replicable amygdala response across an array of imaging genetics studies (Dannlowski et al., 2016; Nikolova et al., 2014; Redlich et al., 2015b; Schneider et al., 2018). The paradigm, which utilized a face-processing task (faces with anger or fear expressions), alternating with a sensorimotor control task was conducted as described previously (Dannlowski et al., 2011; Redlich et al., 2015a) (see Supplementary Information and Figure S3). Functional images were realigned and unwarped, spatially normalized to standard Montreal Neurological Institute (MNI) space, and smoothed using a Gaussian kernel (6 mm Full Width Half Maximum; FWHM). Due to movement effects 16 individuals had to be excluded from further analysis (exclusion criterion >3 mm and/or 3°). Onsets and durations of the two experimental conditions (faces and shapes) were modeled using a canonical hemodynamic response function in the context of the general linear model (GLM). The model was corrected for serial correlations and a high-pass filter of 128 s was applied to reduce low frequency noise. An individual contrast image was generated in each fixed-effects first-level analysis comparing activation in response to fear-relevant faces with the control condition as baseline. The resulting contrast images were further used in second-level random-effects group analyses.

2.4 | Statistical analysis

Statistical analyses were conducted using SPSS (Version 23.0, IBM, Chicago, IL) with a consequent cut-off *p*-value of .05.

2.5 | Principal component analysis of BDNF methylation

Due to high intercorrelations of CpG sites (please see Table S2), leaving the statistical problem of multicollinearity, a principal component analysis (PCA) was used for data reduction of the six CpG sites, using components with an eigenvalue >1 as criteria. The PCA yielded two principal components (PC) with an eigenvalue >1. PC1 explained 49.8% and PC2 explained 19.3% of the total variance. All six CpG sites had factor loadings <0.35 (Table S1). To facilitate the analysis, both PCA factors were extracted to represent the overall *BDNF* exon IX methylation in the dataset. All assessed PCs and methylation values of the CpG sites were correlated with each other and checked for significant associations with the *BDNF* Val^{66}Met polymorphism, age, gender, and BMI.

2.6 | BDNF methylation and genotype

According to our first study aim, we performed a multivariate analysis of variance (MANOVA) including both *BDNF* methylation PCA factors (PC1 and PC2) as dependent variables and the Val^{66}Met polymorphism (val/val vs. val/met and met/met) as independent variable. Further, gender, age, and BMI where included as variables of no interest. As additional analyses, we investigated the associations between BDNF methylation, BMI, age and gender, each controlling for the remaining variables. In addition, the above applied model were also calculated for rs1519480 (CC and CT vs. TT) and rs12273363 (CC and CT vs. TT), according to the haplotype analysis (see supplementary material). Continuous predictors were standardized in order for their coefficients to be more comparable.

2.7 | fMRI analyses

Functional MRI analyses were conducted using Statistical Parametric Mapping (SPM12, http://www.fil.ion.ucl.ac.uk/spm). In order to investigate the effects of *BDNF* methylation and the Val^{66}Met polymorphism (*BDNF*-Val^{66}Met) on amygdala reactivity (study aim 2), an ANCOVA was calculated using a SPM full factorial model, with genotype (val/val vs. val/met and met/met) and *BDNF* methylation as independent variables, again including sex, age, and BMI as covariates of no interest. Again, the above applied model were also calculated for rs1519480 (CC and CT vs. TT) and rs12273363 (CC and CT vs. TT). Given previous studies associating BDNF and the amygdala (Montag et al., 2008; Sagarkar et al., 2017) and its central role in emotion processing (Phelps & LeDoux, 2005), all calculations were restricted to the bilateral amygdala as defined by Tzourio-Mazoyer et al. (2002) using an anatomical mask created with the Wake Forest University (WFU) Pick Atlas (Maldjian, Laurienti, Kraft, & Burdette, 2003). However, to cover for potential nonhypothesized effects on other brain regions, we also conducted additional explorative whole brain analyses. Significance thresholds for multiple testing were obtained at the cluster-level by threshold-free cluster enhancement as a nonparametric approach, which is implemented in the TFCE-toolbox (http://dbm.

neuro.uni-jena.de/tfce, Version 140). We consequently established a conservative FWE-corrected threshold of $p < .05$ obtained by 1,000 permutations per test. For each participant the mean contrast values of significant clusters were extracted from SPM and further analyzed in SPSS. The post hoc sensitivity analysis (http://www.gpower.hhu.de/) revealed that the sample size is suitable to detect (1) strong interaction or group based main effects ($\eta^2 = .30$, $f = .66$), small to medium effects for post hoc group comparisons ($\eta^2 = .04$, $d = .46$) and small effects for correlational main effects ($r = .09$) with good statistical power ($\alpha = .05\%$, $1 - \beta = 80\%$).

2.8 | Personality traits

According to our third study aim, we investigated associations between *BDNF* methylation, *BDNF*-Val^{66}Met, limbic brain function with the TPQ-scales using partial correlations controlling for age, gender, and BMI.

3 | RESULTS

3.1 | BDNF methylation rates

Analyses of individual *BDNF* methylation profiles showed different methylation rates for all CpG sites (Table S1). The principal components analysis revealed two principle components, which accounts for 49.8% (PC1) and 19.3% (PC2) of the variation in methylation across the *BDNF* CpG sites. Correlations analyses and Mann–Whitney *U* tests revealed significant associations of high *BDNF* CpG methylation rates with *BDNF*-^{66}Met, high age and high BMI values (see Tables S2 and S3).

3.2 | BDNF methylation and genotype

Using Pillai's trace, the MANOVA predicting *BDNF* methylation PC1 and PC2 revealed a significant effect of *BDNF*-Val^{66}Met ($V = .06$, $F_{[2,167]} = 5.17$, $p = .007$, $\eta^2 = .062$) and BMI ($V = .04$, $F_{[2,167]} = 3.37$, $p = .037$, $\eta^2 = .038$), while the *BDNF*-rs12273363 ($p = .414$), *BDNF*-rs1519480 ($p = .315$), gender ($p = .753$), and age ($p = .143$) were not significantly associated with *BDNF* methylation PC1 and PC2. Separate univariate ANOVAs revealed a significant effect of *BDNF*-Val^{66}Met on *BDNF* methylation PC2 (F[1,168] = 6.80, $p = .01$, $\eta^2 = .043$), indicating higher *BDNF* methylation in the group of *BDNF*-Met carrier (Figure 1). An additional ANCOVA separating *BDNF*-Val^{66}Met into three groups (val/val, val/met, met, met) confirmed the main effect of BDNF genotype on BDNF methylation PC2 ($F_{[2,167]} = 2.67$, $p = .24$). Post hoc analysis showed that this result was driven by the heterozygous group compared to the BDNF val/val homozygous group ($T_{[1,63]} = 2.88$, $p = .004$) while the BDNF met/met homozygous group showing neither a significant difference to BDNF val/val homozygous nor BDNF heterozygous, which might be caused by the small sample size of $n = 8$ met homozygotes. Further, BMI showed significant effects on *BDNF* methylation PC1 (F[1,168] = 5.13, $p = .025$, $\eta^2 = .029$) and on PC2 (F[1,168] = 3.90, $p = .05$, $\eta^2 = .023$), with high BMI values associated with low *BDNF* methylation.

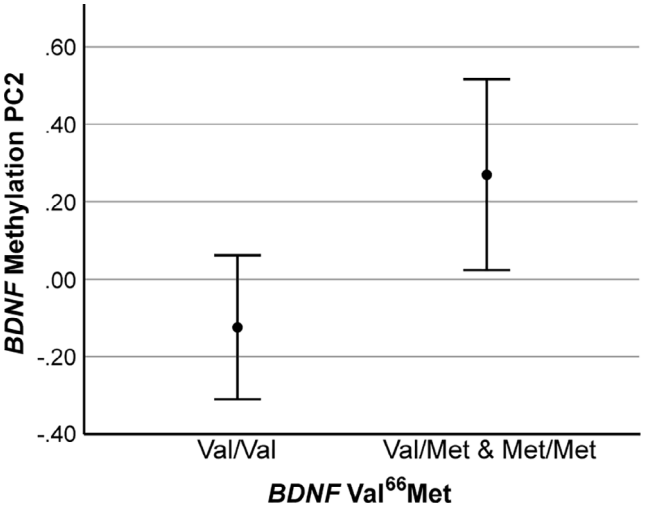

FIGURE 1 Effects of *BDNF* Val^{66}Met polymorphism on *BDNF* PC2 methylation. *BDNF* PC2 methylation was significantly higher in the group of *BDNF*-Met allele carrier (F[1,168] = 6.80, $p = .01$). Error bars depict 95%-CIs. Abbreviations: *BDNF*, brain derived neurotropic factor; PC2, principal component factor 2

3.3 | fMRI results

The analysis of brain function revealed a significant positive association of *BDNF* methylation PC1 ($x = 34$, $y = 0$, $z = -26$, $t_{(166)} = 3.00$, TFCE = 42.39, $p_{(FWE)} = .045$, $k = 6$, Figure 2, $\eta^2 = .048$) and PC2 ($x = 30$, $y = -2$, $z = -24$, $t_{(166)} = 2.75$, TFCE = 56.95, $p_{(FWE)} = .031$, $k = 76$, $\eta^2 = .041$ Figure 2) with right amygdala reactivity to negative emotional faces. Including *BDNF-Val^{66}Met* as covariate to the model does not significantly alter the results for PC1 ($t_{(165)} = 2.94$, TFCE = 41.99, $p_{(FWE)} = .046$) and PC2 ($t_{(165)} = 2.73$, TFCE = 56.34, $p_{(FWE)} = .035$) indicating these association is independent from *BDNF-Val^{66}Met genotype*. To determine the amygdala subregions, the SPM Anatomy toolbox (Eickhoff et al., 2005) was used. According to the implemented probabilistic cytoarchitectonic (Amunts et al., 2005), the cluster were located in the basolateral amygdala. Neither a significant main effect of *BDNF*-Val^{66}Met nor an interaction effect with *BDNF* methylation PC1 or PC2 emerged. As for *BDNF*-Val^{66}Met no significant main effects or interaction effects with PC1 or PC2 emerged for rs1519480 and rs12273363. All abovementioned associations with amygdala reactivity were not significantly affected by sex, BMI and age, as analyzed by subsequent multiple regression with *t*-values ranging from $t = -1.90$ to $t = 1.47$. The whole-brain analyses did not reveal any additional significant results.

3.4 | Personality traits

The analysis of TPQ revealed a significant negative correlation of TPQ-NS with *BDNF* methylation PC1 ($r = -.21$, $p = .007$), and a

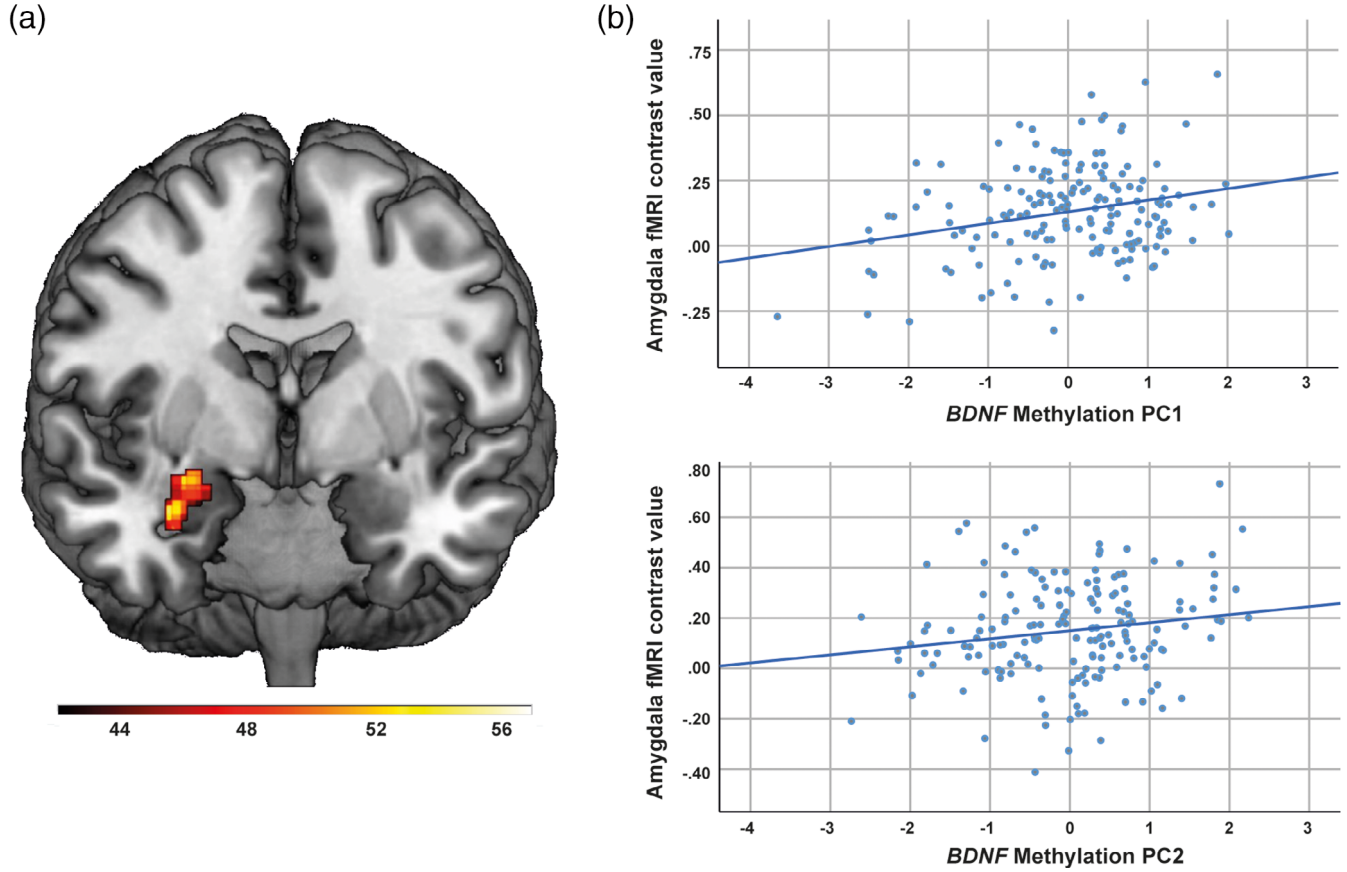

FIGURE 2 Association of *BDNF* methylation and amygdala activity. (a) Coronal view (Montreal-Neurological-Institute coordinate y = 0) depicts the positive association between *BDNF* methylation and amygdala activity in reaction to negative emotional stimuli (PC1 = TFCE = 42.39, $p_{(FWE)}$ = .045, *k* = 6; PC2 = TFCE = 56.95, $p_{(FWE)}$ = .031, *k* = 76). Color bar: TFCE value. (b) Scatter plots depicting the positive correlations of extracted amygdala cluster values and *BDNF* methylation for PC 1 (above, *r* = .25, *p* < .001) and PC2 (below, *r* = .16, *p* < .016). Abbreviations: *BDNF*, brain derived neurotropic factor; PC, principal component factor

TABLE 2 Partial correlation coefficients for TPQ with *BDNF* methylation and amygdala cluster associated with *BDNF* methylation (df = 168)

	BDNF methylation				Amygdala			
	PC1		PC2		Cluster PC1		Cluster PC2	
TPQ	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value
HA	.15	.059	.08	.319	.01	.861	.02	.823
NS	−.21	.007*	−.08	.317	−.17	.027*	−.15	.048
RD	−.13	.094	.51	.505	−.19	.012*	−.12	.131

Note: Correlations were controlled for gender, age and BMI, *p*-values are two-tailed. A correction for multiple comparisons using FDR correction following the Benjamini–Hochberg procedure (Benjamini and Hochberg, 1995) revealed that all comparisons below an FDR-corrected threshold of critical *p* < .012 (*q* = 0.05) are considered significant (*). Abbreviations: *BDNF*, brain derived neurotropic factor; HA, harm avoidance; NS, novelty seeking; PC, principal component factor; RD, reward dependence; TPQ, Tridimensional Personality Questionnaire.

trend for a positive correlation of TPQ-HA and *BDNF* methylation PC1 (*r* = .15, *p* = .059). Further, TPQ-NS was associated with the amygdala reactivity in cluster of *BDNF* methylation PC1 (*r* = −.17, *p* = .027) and by tendency in PC2 (*r* = −.15, *p* = .048). *BDNF*-Val^{66}Met was not associated with any of the TPQ-scales. For details, please see Table 2.

4 | DISCUSSION

In the present study, we highlight a critical role of *BDNF* methylation in human amygdala response to negative emotional stimuli, whereby high *BDNF* methylation rates were for the first time shown to be associated with a high reactivity in the amygdala. Moreover, high *BDNF*

methylation and high amygdala reactivity were associated with low novelty seeking. Although *BDNF* methylation was partly influenced by Val^{66}Met, with Met allele carrier revealing higher *BDNF* methylation, there was no interaction or main effect of the Val^{66}Met polymorphism on amygdala reactivity.

Our results add evidence to the hypothesis that epigenetic modification of *BDNF* methylation might play an important role in amygdala functions, presumably independently from *BDNF* genotype. Recently, a study in rodents showed that DNA hypermethylation of the *BDNF* exon IV and IX in the amygdala resulted in a down-regulation of *BDNF* mRNA levels in the amygdala and increased anxiety-like behaviors (Sagarkar et al., 2017). In humans, *BDNF* exon IV methylation in post-mortem brain tissue of patients who committed suicide revealed as well an inverse correlation of methylation and *BDNF* mRNA levels (Keller et al., 2010). Based on our data and previous research the question arises how epigenetically down-regulated *BDNF* levels could enhance amygdala reactivity. One possibility is that the *BDNF* down-regulation directly alters synaptic plasticity and fear learning via modifying amygdaloid synaptic strength and dendritic spines, thereby promoting amygdala excitability and environmental sensitivity (Agassandian, Gedney, & Cassell, 2006; Ehrlich & Josselyn, 2016). Another way is the broad modification of other neurotransmitter systems via *BDNF*, like the GABAergic, glutamatergic, serotonergic, and neuropeptide Y system (Barnea & Roberts, 2001; Mamounas, Blue, Siuciak, & Altar, 1995; Matsumoto et al., 2006), which in turn have shown to modulate amygdala reactivity (Herman, Contet, Justice, Vale, & Roberto, 2013; Kirson, Oleata, Parsons, Ciccocioppo, & Roberto, 2018; Wood et al., 2016). Together, high *BDNF* exon XI methylation may lead to a downregulation of amygdala *BDNF* mRNA levels, which in turn could have modified the amygdala reactivity. Evidence supporting this hypothesis can also be derived from previous studies. First, higher *BDNF* DNA methylation has also been found in MDD patients (Kang et al., 2013), a diagnosis frequently associated with high amygdala reactivity (Redlich et al., 2017; Redlich et al., 2018; Stuhrmann et al., 2013). Second, it has been shown that the intake of epigenetic modifying medicaments like citalopram increases peripheral *BDNF* levels (Lopez et al., 2013) which goes along with reduced amygdala reactivity (Murphy, Norbury, O'Sullivan, Cowen, & Harmer, 2009). Third, a reduction of *BDNF* methylation could be achieved by psychotherapy (Perroud et al., 2013), which also results in decreased amygdala reactivity (Straub et al., 2015). Since altered amygdala reactivity has multiple times been associated with psychiatric disorders (Gaffrey et al., 2011; Goodman et al., 2014; Schneider et al., 2018; Schumann, Bauman, & Amaral, 2011), our data suggests high *BDNF* exon IX methylation as a potential risk factor for psychiatric disorders. However, since correlations do not prove causality, a direct link between human *BDNF* mRNA/protein levels, methylation, amygdala reactivity and psychiatric disorders is still missing, demanding further research. The fact that our results mainly involve the right amygdala is in line with previous studies. While early reviews particularly summarized a preponderance of left amygdala activations over right amygdala activations in functional neuroimaging studies of emotion processing (Baas, Aleman, & Kahn, 2004), more recent reviews suggest a general right hemisphere dominance for all kinds of emotions, and, more specifically, a critical role of the right amygdala in the early assessment of emotional stimuli (Gainotti, 2019). This is in line with a study that confirms a right amygdala's key role in right anterior hemisphere cross-talk in subjects who are likely more stress-sensitive in general, and that high HA in particular is associated with a stronger right amygdala resting state functional connectivity with the dorsomedial prefrontal cortex, which is implicated in negative affect regulation (Baeken et al., 2014).

Furthermore, we found significant negative associations of NS with *BDNF* methylation and amygdala reactivity. Individuals with high NS are described as enthusiastic, impulsive and highly explorative to novel and rewarding situations. In animal models, low novelty seeking behavior is associated with higher vulnerability to depression-like behavior (Stedenfeld et al., 2011), while high novelty seeking is associated with resilience to negative effects of early life stress (Clinton, Watson, & Akil, 2014). Our findings align well with a study showing higher BDNF protein levels along with lower *BDNF* histone methylation in high NS compared to low NS rats (Duclot & Kabbaj, 2013). At the neurofunctional level, NS was negatively correlated with the right amygdala reactivity in the fMRI clusters associated with *BDNF* methylation. This is in line with studies showing amygdala affective processing might be linked with the temperament dimensions (Baeken et al., 2014), and that selective amygdala lesions in monkeys have been shown to increase NS personality traits like exploration and excitability (Machado, Kazama, & Bachevalier, 2009). In summary, the association of high *BDNF* methylation, high amygdala reactivity and low novelty seeking underlines again the possible role of *BDNF* methylation in psychiatric disorders. However, determining the underlying directions of the relations between *BDNF* methylation, amygdala reactivity, and NS cannot be accomplished based on our data and must await further research. Previous research indicates that other factors like brain injuries (Sagarkar et al., 2017), early caregiving environment and childhood maltreatment (Doherty, Forster, & Roth, 2016; Perroud et al., 2013), and the Val^{66}Met polymorphism (Mill et al., 2008) might also determine *BDNF* methylation.

Furthermore, we found a negative association between BMI values and BDNF methylation. In addition to BDNF key roles in neuronal survival and development, it is important for the control of body weight (Xu & Xie, 2016). These results are in line with a previous study reporting BDNF hypomethylation in currently obese individuals compared to successful weight loss maintainers and normal weight individuals (Huang et al., 2015), and support the suggestion that the association between high BMI values and low BDNF methylation might reflect an epigenetic adaption of BDNF mRNA levels to the current nutrition status. However, since body weight was not a primary target in our study and we did not collect detailed nutrition status of our participants, this only provides small evidence and further research is needed to investigate the epigenetic BDNF regulation of energy balance in more detail.

The analysis of the Val^{66}Met polymorphism revealed several implications. First, the association between the *BDNF* Val^{66}Met polymorphism and methylation is in line with an earlier enrichment microarray

analysis from frontal-cortex brain tissue (Mill et al., 2008). However, while Mill et al. (2008) found a higher methylation in exon IX of two CpG sites (bp172 and bp112) in Val homozygotes, we found a higher methylation of two CpG sites (bp166 and bp209) in Met allele carriers. Based on the Met allele (T instead of C) Met homozygotes do not have the CpG site bp172, which likely explain the hypomethylation compared to Val homozygotes in the data of Mill et al. and the undetectable methylation for Met allele carriers in our data. The further differences could rely on tissue specific methylation rates, although studies reported similarity between blood and brain methylation rates (Klein et al., 2011). In addition, one third of the investigated participants by Mill et al. (2008) were patients with schizophrenia and bipolar disorder, which might have resulted in a different average methylation compared to healthy controls (Mill et al., 2008). As described above, the higher *BDNF* methylation in ^{66}Met carriers might lead to lower *BDNF* mRNA levels (Keller et al., 2010; Sagarkar et al., 2017). Previous studies have likewise reported a reduction of activity-dependent *BDNF* release in Met allele carriers (Chen et al., 2005; Chen et al., 2006; Egan et al., 2003). Based on these results the hypothesis arises, that the inconclusive associations of the Val^{66}Met polymorphism with psychiatric disorders and anxiety-related endophenotypes (Frustaci et al., 2008) might be explained by an additional impact of epigenetic influences on *BDNF* expression (Chen et al., 2015). Second, we did not find significant associations between Val^{66}Met polymorphism and personality traits, which is consistent with previous negative findings in the literature (Frustaci et al., 2008; Montag, Basten, et al., 2010a; Wei et al., 2016). Finally, we did not find any genotype effect on amygdala reactivity. Although several fMRI studies reported associations of the BDNF val^{66}met genotype with amygdala reactivity in response to emotional stimuli (Lau et al., 2010; Montag et al., 2008; Perez-Rodriguez et al., 2017), the results of these studies were limited by the single SNP approach, restricted generalizability of findings for females or MDD patients, and, in general, modest sample sizes. However, though the current study considered eight BDNF SNPs in a—relatively—large sample, we do not find a significant effect of BDNF genotype. Therefore, our findings add more evidence to Lau et al. (2010) results, revealing no direct Val^{66}Met polymorphism effect on amygdala reactivity in healthy controls (Lau et al., 2010). Nevertheless, more research is required to analyze the relations between BDNF genotype, methylation and brain functions to create a clearer picture of the underlying relations, preferably using the GWAS approach.

Several limitations should be considered in interpreting the results. First, DNA methylation was measured using whole EDTA-blood. Inter-subject heterogeneity in blood cell type proportions might potentially confound methylation levels in our study (Jaffe & Irizarry, 2014). Furthermore, since determination of *BDNF* methylation in the brain of live patients is impractical, a direct correlation between *BDNF* methylation levels in the blood and brain cannot be assured. However, previous work indicates that *BDNF* can cross the blood–brain barrier, leading to detectable changes in peripheral blood (Hing, Sathyaputri, & Potash, 2017). Positive correlations between whole-blood BDNF levels and hippocampal *BDNF* in rats and pigs were observed (Klein et al., 2011), underlining peripheral BDNF measurements as a useful predictor of neuronal BDNF appearance (Hing et al., 2017; Kundakovic et al., 2015). The not significant *BDNF* polymorphism effects on the amygdala and personality trait level might be due to the low amount of Met/Met allele (n = 8) carriers and the resulting combination of Met/Met and Val/Met carriers into one group. Besides, influences of other *BDNF* exons, histone modifications, and functional polymorphisms could be biologically relevant and potentially confound our results. *SLC6A4*, for instance, has frequently been shown to interact with BDNF on behavioral, transcriptional, and epigenetic levels (Ignácio, Réus, Abelaira, & Quevedo, 2014). Last but not least, the cpg site (bp172) that includes the BDNF Val^{66}Met SNP (rs6265) was excluded from our analysis, since it is only available in a part of subjects (BDNF met/met homozygotes and 50% of heterozygotes) leaving not enough power. In addition, other confounders like smoking status, physical exercises or stress could have influenced the *BDNF* methylation status and amygdala reactivity and should be taken into account for future studies. Finally, although we applied a computational and visual inspection of fMRI movement outliers and additionally used realignment, subtle movement effects cannot be completely ruled out.

In conclusion, we showed an association of *BDNF* methylation, amygdala reactivity and personality traits in humans, highlighting the multidimensional relations among genetics, epigenetics, and neuronal functions. Our data adds evidence to the hypothesis that epigenetic modifications of *BDNF* can result in an endophenotype associated with anxiety and mood disorders. Future more comprehensive epigenetic analyses are needed to examine further environmental, genetic, and epigenetic factors involved in the association of *BDNF* methylation and amygdala reactivity in detail.

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DECLARATION OF INTEREST

VA is member of advisory boards and/or gave presentations for the following companies: Astra-Zeneca, Janssen-Organon, Lilly, Lundbeck, Servier, Pfizer, Otsuka, Trommsdorff, and Wyeth. He also receives funds from the German Ministry of Education and Research (BMBF) and from the European Union (EU-FP7). BTB is member of advisory boards, received funding and/or gave presentations for the following companies: AstraZeneca, Lundbeck, Pfizer, Servier, and Wyeth. He receives funding from the National Health and Medical Research

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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