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Interaction between BDNF Val66Met and Dopamine Transporter Gene Variation Influences Anxiety-Related Traits

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The involvement in neural plasticity and the mediation of effects of repeated stress exposure and long-term antidepressant treatment on hippocampal neurogenesis supports a critical role of brain-derived neurotrophic factor (BDNF) in the pathophysiology of affective and other stress-related disorders. A previously reported valine to methionine substitution at amino-acid position 66 (BDNF Val66Met) seems to account for memory disturbance and hippocampal dysfunction. In the present study, we evaluated the impact of the BDNF Val66Met polymorphism on individual differences in personality traits in a sample of healthy volunteers in relation to other common gene variants thought to be involved in the pathophysiology of affective disorders, such as the serotonin transporter promoter polymorphism (5-HTTLPR) and a variable number of tandem repeat polymorphism of the dopamine transporter gene (DAT VNTR). Personality traits were assessed using the NEO personality inventory (NEO-PI-R) and Tridimensional Personality Questionnaire (TPQ). There was a significant DAT VNTR-dependent association between NEO-PI-R Neuroticism and the BDNF Val66Met polymorphism. Among individuals with at least one copy of the DAT 9-repeat allele, carriers of the BDNF Met allele exhibited significantly lower Neuroticism scores than noncarriers. This interaction was also observed for TPQ Harm Avoidance, a personality dimension related to Neuroticism. Our results support the notion that allelic variation at the BDNF locus—in interaction with other gene variants—influences anxiety- and depression-related personality traits.

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INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is involved in a variety of trophic and modulatory effects that include an essential function in the development and plasticity of dopaminergic, serotonergic, and other neurons (Bonhoeffer, 1996; Lu and Chow, 1999; Poo, 2001). Reduced expression of BDNF modifies synaptic plasticity resulting in specific alterations in spatial learning and memory processes, anxiety-related behavior, and motor activity in the knock-out mouse model (McAllister *et al*, 1999; Kernie *et al*, 2000; Carter *et al*, 2002; Tyler *et al*, 2002; Yamada *et al*, 2002). However, targeted inactivation of the BDNF receptor, TrkB, leads to neuronal loss and cortical degenerative changes (Vitalis *et al*, 2002; Xu *et al*, 2000). In addition, BDNF mediates the effects of repeated stress exposure and long-term antidepressant treatment on neurogenesis and neuronal survival in the hippocampus (D'Sa and Duman, 2002; Rasmusson *et al*, 2002). These findings converge with impaired hippocampal plasticity in depression reflected by a reduced hippocampal volume and hippocampus-related memory deficiency, which supports that BDNF plays a critical role in the pathophysiology of affective and other stress-related disorders (Duman *et al*, 1997, 2001; Garcia, 2002; Hull, 2002; McEwen and Magarinos, 2001).

In fact, the experience of stressful life events is neither necessary nor sufficient for the development of depression or anxiety disorders. Liability to these disorders is, therefore, more likely to arise from a complex interaction of stress exposure with a variety of vulnerability factors including anxiety-related personality traits, such as Neuroticism (Costa and McCrae, 1992; Eysenck and Eysenck, 1985), Harm Avoidance (Cloninger, 1987), or Negative Emotionality (Watson and Tellegen, 1985). The spectrum of these higher-order personality traits refers to a basic concept comprising lower-order traits like stress reactivity, anxiety, depressiveness, or hostility. The finding that approximately 55% of the genetic liability of major depression is shared with Neuroticism (Kendler *et al*, 1993) renders it plausible that some of the same genes that modulate the disposition to depression also affect individual differences in anxiety-related traits.

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Sen *et al* (2003) reported the association between Neuroticism and a valine to methionine substitution at amino-acid position 66 (BDNF Val66Met) located in the N-terminal sequence upstream of the mature BDNF protein. Individuals with the rare BDNF Met/Met genotype showed significantly lower Neuroticism scores, which led to the conclusion that the Val allele may increase the risk for anxiety and depression spectrum disorders. This notion is further supported by several recent family-based studies revealing a preferential transmission of the BDNF Val allele to bipolar individuals (Neves-Pereira *et al*, 2002; Sklar *et al*, 2002; Geller *et al*, 2004; Lohoff *et al*, 2005).

The aim of our study was, therefore, to confirm the association between the BDNF Val66Met polymorphism and Neuroticism, and furthermore, to explore possible interactions of this polymorphism with other common gene variants thought to be involved in the pathophysiology of stress-related and affective disorders. Based on the evidence of a reciprocal modulatory interaction of BDNF, serotonin, and dopamine (Vollmayr *et al*, 2000; Goggi *et al*, 2002; Canals *et al*, 2001; Küppers and Beyer, 2001), and of this interaction in the pathophysiology of depression (Meltzer, 1990; Willner *et al*, 1991) and stress disorders (Vermetten and Bremner, 2002), we focused specifically on the variation in genes influencing the functionality of the serotonergic and dopaminergic system. Hence, interaction effects of the BDNF Val66Met polymorphism were investigated with the length variation in the transcriptional control region of the serotonin transporter (5-HTT, *SLC6A4*) gene (5-HTT linked polymorphic region, 5-HTTLPR) and with the variable number of tandem repeat polymorphism in the 3′-untranslated region of the dopamine transporter gene *SLC6A3* (DAT VNTR), which both have previously been reported to modify expression of the respective gene. 5-HTTLPR seems to play a role in the modulation of Neuroticism (Lesch *et al*, 1996; Greenberg *et al*, 2000) and in several neuropsychiatric disorders including affective disorders (Lesch, 2001). Furthermore, serotonin transporter function was demonstrated as being influenced by BDNF in a 5-HTTLPR genotype-dependent fashion (Mössner *et al*, 2000). The DAT VNTR has been less intensively investigated, although it had been reported previously as being associated with post-traumatic stress disorder (PTSD) (Segman *et al*, 2002). It seems, therefore, reasonable to examine potential interactive influences of these polymorphisms and BDNF on individual differences in Neuroticism and related personality traits.

PARTICIPANTS AND METHODS

Participants and Procedures

Two hundred seventy-two healthy volunteers of German ethnicity (203 females and 69 males; mean age: 21.9 ± 3.8 years, age range: 18–41 years) were recruited from the student body and staff at the Dresden University of Technology. The study was carried out in accordance with the Declaration of Helsinki. All participants gave written informed consent, and 200 μl of blood from earlobes was obtained. Participants completed the German versions of the Revised NEO Personality Inventory (NEO-PI-R; Ostendorf and Angleitner, 2003) and the Tridimensional Personality Questionnaire (TPQ; Weyers *et al*, 1995). The NEO-PI-R is based on the five-factor model of personality (Costa and McCrae, 1992). It consists of 241 Likert-type items and assesses individual differences in 30 personality facets, which can be aggregated to the five personality factors—Neuroticism, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness. The TPQ is based on Cloninger's neurobiological theory of personality (Cloninger, 1987). Its 100 items assess individual differences along the temperament dimensions: Novelty Seeking, Harm Avoidance, and Reward Dependence. Each of the three temperament scales is comprised of four subscales, the Reward Dependence subscale Persistence mostly being considered as a separate fourth temperament factor (Cloninger *et al*, 1993). The German versions of the NEO-PI-R and the TPQ exhibit acceptable psychometric properties (Ostendorf and Angleitner, 2003; Weyers *et al*, 1995).

Genotyping

DNA was extracted from EDTA blood using the QIAamp Blood Kit (Qiagen, Hilden, Germany).

For the *BDNF* gene, the G→A single nucleotide polymorphism (SNP) coding for the Val66Met substitution was genotyped following a modified protocol by Sen *et al* (2003) and Mössner *et al* (2005). A 274-bp polymerase chain reaction (PCR) product containing the SNP was amplified by PCR using the following reaction mix: 20 ng of genomic DNA in 75 mM Tris-HCl (pH 9.0), 20 mM ammonium sulfate, 0.01% Tween 20, 1.5 mM magnesium chloride, 0.4 μM of each of the primers, BDNF-for (5′-AAA GAA GCA AAC ATC CGA GGA CAA G) and BDNF-rev (5′-ATT CCT CCA GCA GAA AGA GAA GAG G), 0.4 mM dNTP, and 1 U *Taq* polymerase. After an initial denaturation for 5 min at 95°C, 35 cycles of denaturating at 95°C for 30 s, annealing at 55°C for 40 s, and extension at 72°C for 50 s were performed, followed by a final extension at 72°C for 5 min. PCR products were digested with *Nla*III. The undigested PCR product carries the A variant, whereas the digested product with three fragments of 57, 77, and 140 bp contains the G allele.

For the DAT gene, the 40-base-pair VNTR located in the 3′-untranslated region of the DAT cDNA was amplified from genomic DNA using 5 U of *Taq* DNA polymerase. After an initial denaturation for 3 min at 95°C, 35 cycles of denaturing at 93°C for 45 s, annealing at 67.5°C for 45 s, and extension at 72°C for 45 s were performed in the presence of primers 5′-TGT GGT GTA GGG AAC GGC CTG AG-3′ and 5′-CTT CCT GGA GGT CAC GGC TCA AGG-3′, followed by a final extension at 72°C for 3 min. PCR amplification was carried out in a final volume of 25 μl consisting of 80 ng of genomic DNA, 250 μM of each deoxyribonucleotide, 10 pmol of sense and antisense primers, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 1.5 mM $MgCl_2$.

For the 5-HTT gene, the 5-HTTLPR was genotyped according to a previously published protocol (Lesch *et al*, 1996).

BDNF allele frequencies were 16.9% for the A (Met) allele and 83.1% for the G (Val) allele, and BDNF genotype frequencies were 3.3% for Met/Met, 27.2% for Val/Met, and

69.5% for Val/Val genotypes. 5-HTTLPR allele frequencies were 61.2% for the l allele and 38.4 for the s allele, and genotype frequencies were 36.0% for l/l, 50.5% for l/s, and 13.2% for s/s (genotype data of one participant were missing). DAT VNTR allele frequencies were 25.2% for the 9-repeat and 74.8% for the 10-repeat, DAT VNTR genotype frequencies were 7.0% for 9/9, 36.4% for 9/10, and 56.6% for 10/10 genotypes. All genotypes were in Hardy–Weinberg equilibrium (χ^2-tests, all $p \geqslant 0.271$). For subsequent statistical analyses focusing on gene–gene interactions, the BDNF, 5-HTTLPR, and DAT VNTR genotypes were dichotomized to enhance statistical power. The groups of individuals with the BDNF Met/Met and Val/Met genotypes were combined based on the Met-related impairments observed by Egan *et al* (2003). Likewise, 5-HTTLPR genotypes were grouped according to the presence (S) or absence (L) of the s allele by reference to the observed dominant effect of the s allele (Lesch *et al*, 1996). DAT VNTR genotypes were dichotomized for the presence (9+) or absence (9−) of the DAT 9-repeat allele based on the differential DAT expression in the presence of the 9-repeat allele (Fuke *et al*, 2001; Mill *et al*, 2002).

Statistical Analyses

All analyses were carried out using SPSS 9.0.1 (SPSS Inc., Chicago, IL, USA). Association tests were performed by means of analyses of variance (ANOVA) with the polymorphisms as independent variables and NEO Neuroticism or its facet scales as dependent variables. The discriminant validity of possible results was assessed by means of an analysis of variance with the other four NEO domain scales as dependent variables, and convergent validity (ie stability of potential effects remained across measurement methods) was assessed by repeating the respective ANOVAs with TPQ Harm Avoidance as the dependent variable. Age and gender were included as covariates in the analyses to control for possible confounding effects of demographic variables. In all analyses, there was a significant covariate effect of gender, with women scoring higher on Neuroticism and on its facet scales (except for N5—Impulsivity), as well as on Harm Avoidance. Similarly, a significant covariate effect of age was observed in some of the analyses, with younger participants exhibiting higher Neuroticism scores. Uncorrected two-tailed *p*-values are reported throughout the manuscript, and where applicable, Bonferroni-corrected significance levels are given. Otherwise, the type I error rate is 0.05.

RESULTS

First, we tested the hypothesis that Neuroticism is influenced by the BDNF Val66Met polymorphism using a univariate ANOVA with the BDNF Met/Met *vs* Val/Met *vs* Val/Val genotype as independent variable and the Neuroticism domain score as dependent variable. There was no significant effect of Val66Met on Neuroticism scores ($F_{2,267} = 0.04$, $p = 0.963$). Similarly, when performing a multivariate ANOVA with the six Neuroticism facet scales as dependent variables, neither a multivariate effect (Wilks Lambda, $p > 0.05$) nor the univariate effects of Val66Met on any of the facet scales were observed (all $p > 0.05$). Raw means and standard errors of means of the Neuroticism domain and facet scale scores grouped by BDNF Val66Met genotype, as well as the number of individuals in each group are given in Table 1.

Next, we examined potential interactive effects of BDNF Val66Met, 5-HTTLPR, and the DAT VNTR on Neuroticism using a univariate ANOVA with dichotomized genotypes for Val66Met (Met+ = Met/Met and Val/Met *vs* Met− = Val/Val), 5-HTTLPR (L = l/l *vs* S = l/s and s/s), and DAT VNTR (9+ = 9/9 and 9/10 *vs* 9− = 10/10) as independent variables. Because of limitations in sample size, an unsaturated model was chosen, focusing on the three main effects and the three two-way interactions while neglecting the three-way interaction. Because six significance tests were performed (three main effects and three two-way interactions), the level of significance was Bonferroni-adjusted resulting in $\alpha' = 0.0083$. No main effects of the three polymorphisms and no significant interactions between Val66Met and 5-HTTLPR, and between 5-HTTLPR and DAT VNTR, respectively, were observed (all $p > 0.05$). However, there was a Val66Met by DAT VNTR interaction effect on Neuroticism, significant at the Bonferroni-adjusted level ($F_{1,262} = 7.23$, $p = 0.008$, $\eta^2 = 0.03$; Figure 1). A *post hoc* ANOVA performed separately for carriers and noncarriers of the DAT 9-repeat allele revealed that among individuals with at least one copy of the DAT 9-repeat allele, carriers of the BDNF Met allele had lower Neuroticism scores compared with noncarriers ($F_{1,114} = 4.28$, $p = 0.041$; $\eta^2 = 0.04$). Among individuals lacking the DAT 9-repeat allele, there were no significant differences in Neuroticism scores between carriers and noncarriers of the BDNF Met allele ($F_{1,150} = 1.95$, $p = 0.165$).

A subsequent analysis with regard to the Neuroticism facets, therefore, focused only on the observed interaction between Val66Met (Met+ *vs* Met−) and DAT VNTR (9+ *vs* 9−) variants. Table 2 gives the respective raw means and

Table 1 Neuroticism Domain and Facet Scale Means and Standard Errors of Mean (SEM) by BDNF Met/Met *vs* Val/Met *vs* Val/Val Genotype

BNDF	*n*	N	N1	N2	N3	N4	N5	N6
Met/Met	9	93.2 (6.1)	15.9 (1.8)	13.3 (1.3)	14.7 (1.7)	16.7 (1.8)	19.6 (1.3)	13.1 (1.6)
Val/Met	74	95.6 (2.8)	17.4 (0.7)	13.3 (0.6)	15.2 (0.7)	18.4 (0.6)	18.2 (0.6)	13.1 (0.6)
Val/Val	189	95.5 (1.9)	17.2 (0.5)	14.3 (0.4)	15.5 (0.5)	17.5 (0.4)	17.9 (0.3)	13.1 (0.4)

N = Neuroticism domain scale score; N1–N6 = Neuroticism facet scale scores; N1 = Anxiety; N2 = Angry Hostility; N3 = Depression; N4 = Self-consciousness; N5 = Impulsiveness; N6 = Vulnerability.

standard errors of means of NEO-PI-R Neuroticism domain and facet scale scores. (Descriptive statistics for the BNDF Val66Met by DAT VNTR full 3×3 genotype grouping as well as for the dichotomized 2×2 and full 3×3 genotype groupings for BDNF by 5-HTTLPR, and for 5-HTTLPR by DAT VNTR, respectively, are available as Supplementary information at the Neuropsychopharmacology website, http://www.nature.com/npp.) The results of the ANOVA showed that the interaction effect of Val66Met and DAT VNTR on Neuroticism observed in the previous analysis was due to univariate interactive effects of the two polymorphisms on N1: Anxiety ($F_{1,266} = 4.63$, $p = 0.032$, $\eta^2 = 0.02$), N3: Depression ($F_{1,266} = 9.14$, $p = 0.003$, $\eta^2 = 0.03$), N4: Self-consciousness ($F_{1,266} = 3.94$, $p = 0.048$, $\eta^2 = 0.02$), and N6: Vulnerability ($F_{1,266} = 10.79$, $p = 0.001$, $\eta^2 = 0.04$). For N2: Angry Hostility, and for N5: Impulsiveness, respectively, the interaction effects were not significant ($p > 0.05$). There were no main effects of BDNF Val66Met and DAT VNTR on Neuroticism facets (all $p > 0.05$). As we performed 18 tests of significance (two main effects and one interaction for six dependent variables), the level of significance was Bonferroni-adjusted resulting in $\alpha' = 0.0028$. At this level, only the interaction effect on N6: Vulnerability remained significant, whereas the effect on N3: Depression was significant at the trend level.

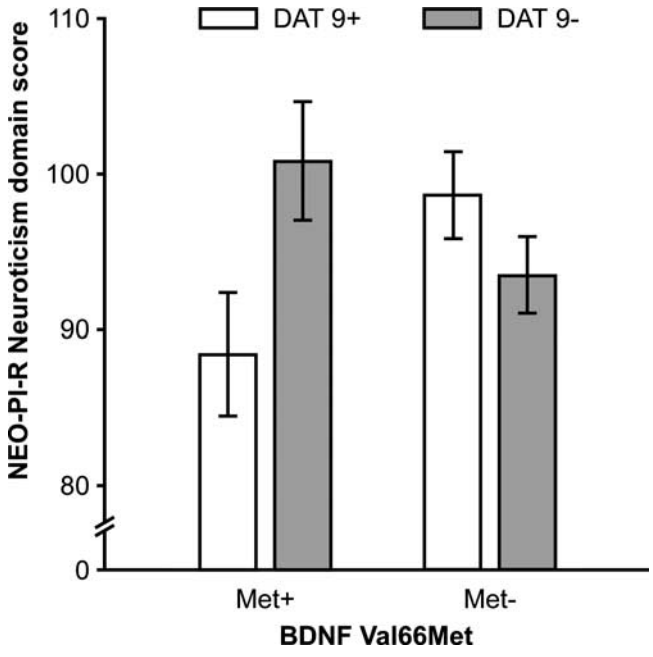

Figure 1 Interaction effect of DAT1 (presence *vs* absence of the 9-repeat allele, 9+ *vs* 9−) and BDNF polymorphism (presence *vs* absence of the Met allele, Met+ *vs* Met−) on NEO-PI-R Neuroticism scores ($p = 0.008$, uncorrected); among carriers of the DAT1 9-repeat allele, carriers of the BDNF Met allele exhibit lower Neuroticism scores compared with noncarriers ($p = 0.041$, uncorrected); among individuals without the DAT1 9-repeat allele, there are no significant differences in Neuroticism scores between the two BDNF genotypes ($p = 0.165$, uncorrected; for details of the ANOVA, see text).

An ANOVA utilizing the four other NEO domain scales (Extraversion, Openness, Agreeableness, and Conscientiousness) as dependent variables demonstrated discriminant validity of the results obtained in the previous analyses. No significant main effects of BDNF Val66Met (Met+ *vs* Met−) or DAT VNTR (9+ *vs* 9−), and no interactions emerged (all $p > 0.05$, all $\eta^2 < 0.01$). Furthermore, an examination of convergent validity employing TPQ Harm Avoidance as dependent variable revealed a significant BDNF Val66Met by DAT VNTR interaction effect ($F_{1,266} = 6.87$, $p = 0.009$, $\eta^2 = 0.03$), whereas again, BDNF Val66Met or DAT VNTR main effects were not detected (all $p \geqslant 0.05$). In the presence of the DAT 9-repeat allele, carriers of the BDNF Met allele had significantly lower Harm Avoidance scores than noncarriers of the Met allele ($F_{1,114} = 6.57$, $p = 0.012$, $\eta^2 = 0.06$).

DISCUSSION

The results of the present study provide evidence for a DAT VNTR-dependent association between NEO-PI-R Neuroticism and the BDNF Val66Met polymorphism, further supporting the notion that allelic variation at the BDNF locus influences anxiety- and depression-related traits, and indicate that these traits are likely to result from an interaction of several or multiple genes. Among individuals with at least one copy of the DAT 9-repeat allele, carriers of the BDNF Met allele exhibit significantly lower Neuroticism scores than noncarriers. Convergent and discriminant validity of this result was verified by showing that this interaction is also observed for TPQ Harm Avoidance, a personality trait similar to Neuroticism, whereas there was no interaction between BDNF Val66Met and DAT VNTR on the other four NEO domain scales. In previous studies, Neuroticism has clearly been found to be associated with both PTSD (Bowman, 1999; Holeva and Tarrier, 2001) and depression (Enns and Cox, 1997).

Table 2 Neuroticism Domain and Facet Scale Means and Standard Errors of Mean (SEM) by BDNF Met+ (=Met/Met and Val/Met) *vs* Met− (=Val/Val) and DAT1 9+ (=9/9 and 9/10) *vs* 9− (=10/10) Genotype

BNDF	DAT1	N	N	N1	N2	N3	N4	N5	N6
Met+	9+	33	89.8 (3.6)	15.9 (0.9)	13.0 (0.7)	13.5 (1.0)	17.5 (0.8)	18.2 (0.7)	11.7 (0.8)
	9−	50	99.9 (3.5)	18.4 (1.0)	13.6 (0.8)	16.5 (0.8)	18.8 (0.7)	18.4 (0.8)	14.3 (0.7)
Met−	9+	65	97.0 (3.0)	17.5 (0.8)	14.3 (0.6)	16.1 (0.8)	17.9 (0.6)	17.4 (0.5)	13.8 (0.6)
	9−	123	94.4 (2.5)	17.0 (0.7)	14.3 (0.5)	15.0 (0.6)	17.2 (0.5)	18.3 (0.4)	12.6 (0.5)

N = Neuroticism domain scale score; N1–N6 = Neuroticism facet scale scores; N1 = Anxiety; N2 = Angry Hostility; N3 = Depression; N4 = Self-consciousness; N5 = Impulsiveness; N6 = Vulnerability.

Although our findings confirm a role of BDNF Val66Met polymorphism in anxiety- and depression-related traits, we did not succeed in replicating the previously reported direct association between the BDNF Met variant and low Neuroticism. This failure may, in part, be due to the smaller sample size of the present study relative to the initial report by Sen *et al* (2003). The present study has a 65% power to detect an effect that accounts for about 2% of the phenotypic variance as estimated from the previously reported data. A problem of statistical power may also be reflected by the lack of evidence for an association between 5-HTTLPR and Neuroticism in our sample. Previous reports have indicated that sample size is critical for the detection of the 5-HTTLPR–Neuroticism association (Lesch *et al*, 1996; Greenberg *et al*, 2000). In the present study on the level of the Neuroticism facets, the BDNF–DAT interaction was significant at the conventional 5% level for the NEO subscales N1: Anxiety, N3: Depression, N4: Self-consciousness, and N6: Vulnerability. It is noteworthy that these facets are exactly the same as those associated with BDNF in the study by Sen *et al* (2003). Although after Bonferroni correction, only the effects on N6: Vulnerability and (at the trend level) N3: Depression were still significant, it has to be noted that Bonferroni correction might be too conservative in analyses with highly inter-related dependent variables.

Beyond Sen's report and our results, there is further evidence of BDNF playing a crucial role in the pathophysiology of anxiety and depression spectrum disorders. Four family-based studies revealed a preferential transmission of the BDNF Val allele to bipolar individuals (Neves-Pereira *et al*, 2002; Sklar *et al*, 2002; Geller *et al*, 2004; Lohoff *et al*, 2005). Recently, Strauss *et al* (2004, 2005) discovered in two independent samples that the Val allele is associated with childhood-onset mood disorder. Concordantly, carriers of the Val/Val genotype showed higher levels in anxiety-related traits and a trend to increased Neuroticism scores (Lang *et al*, 2005). All these investigations found the Val allele connected to a higher degree with anxiety and depressive symptoms. One might, therefore, expect the Val allele to be associated with characteristic memory disturbance and hippocampal dysfunction seen in stress-related disorders. However, Egan *et al* (2003) observed poorer episodic memory, abnormal functional magnetic resonance imaging (fMRI)-assayed hippocampal activation, and lower hippocampal *N*-acetyl aspartate assayed with MRI spectroscopy in human participants carrying the BDNF Met not the Val allele. Hariri *et al* (2003) also revealed a diminished hippocampal engagement and memory impairment associated with the Met allele. Furthermore, Met allele carriers had relative decreases in hippocampal and prefrontal cortical volume, the two brain regions that show especially abundant expression of BDNF (Pezawas *et al*, 2004; Szeszko *et al*, 2005). As a possible explanation at the molecular level, neuronal cultures transfected with the Met allele present abnormal intracellular trafficking and depolarization-induced secretion of BDNF (Egan *et al*, 2003; Chen *et al*, 2004). Unfortunately, these authors did not include personality measures in their study; so currently, this apparent contradiction cannot be resolved and requires further investigation.

It should be mentioned that several authors were unable to affirm that BDNF Val66Met modifies personality dimensions or disposes to mood disorders at all (Hong *et al*, 2003; Kunugi *et al*, 2004; Skibinska *et al*, 2004; Tsai *et al*, 2004). Using the Eysenck Personality Questionnaire, Willis-Owen *et al* (2005) failed to report an association of BDNF Val66Met with Neuroticism, which opposes results found with the TPQ. Contradicting our results, Jiang *et al* (2005) found the BDNF Met allele connected with elevated Harm Avoidance scores. Their varying study design and the use of different diagnostic inventories might explain this divergent outcome. Moreover, population stratification as an artifact could have influenced our positive interaction as BDNF gene variants show heterogeneity across populations.

Nevertheless, further evidence for an involvement of the BDNF Val66Met polymorphism in the modulation of anxiety-related behavior comes from a recent publication (Chen *et al*, 2006). In their study, a variant BDNF mouse (BDNF Met/Met) showed a normal BDNF Met expression in the brain, but a defective BDNF secretion from neurons. Furthermore, the Met allele was associated with increased anxiety-related behaviors of these mice under stressful conditions, an effect that could not be normalized by antidepressant medication with fluoxetine.

In our study, BDNF Val66Met in interaction with a DAT VNTR influences the personality dimensions Neuroticism and Harm Avoidance. The dopamine transporter mediates the uptake of dopamine into neurons, and is a major target for various stimulants. The 40-bp VNTR is located in the 3′-untranslated region adjacent to the polyadenylation site of the human DAT gene. It is likely to give rise to the formation of DNA secondary structure that has the potential to regulate DAT gene transcription, mRNA concentration, protein availability, and, ultimately, transporter function. Evidence that the DAT VNTR contributes to variability in DAT gene expression is ample albeit inconclusive. The 9-repeat allele of the DAT VNTR has been reported to be associated with either lower (Fuke *et al*, 2001; Mill *et al*, 2002) or higher levels of DAT expression (Michelhaugh *et al*, 2001; Miller and Madras, 2002). Furthermore, neuroimaging studies in humans have found the DAT 9-repeat allele associated with either reduced (Heinz *et al*, 2000) or elevated striatal dopamine transporter binding (Jacobsen *et al*, 2000; van Dyck *et al*, 2005), whereas other studies observed no effect at all (Martinez *et al*, 2001; Lynch *et al*, 2003). These apparent discrepancies may arise from a number of reasons, including the existence of allele diversity independent of the length of the DAT VNTR, cell type-specific or developmentally differentiated regulation of the DAT gene transcript levels and stability, as well as gene–gene and gene–environment effects impacting on the DAT gene expression.

Association studies provide considerable evidence that the DAT VNTR 10-repeat allele is associated with attention-deficit hyperactivity disorder (ADHD) (Cook *et al*, 1995; Gill *et al*, 1997; Waldman *et al*, 1998; Daly *et al*, 1999; Barr *et al*, 2001; Curran *et al*, 2001; Chen *et al*, 2003), although several nonreplications have been reported (Palmer *et al*, 1999; Holmes *et al*, 2000; Todd *et al*, 2001; Langley *et al*, 2005). *In vivo* studies using single photon emission computed tomography (SPECT) show an increased density of striatal DAT in ADHD subjects compared with controls (Dougherty *et al*, 1999; Dresel *et al*, 2000; Krause *et al*, 2000, 2003). Cheon *et al* (2005) found a higher DAT density in the

basal ganglia of ADHD children with VNTR 10/10 genotype, suggesting that there might be an association between the VNTR genotype and DAT density.

Furthermore, there is strong evidence for a role of DAT VNTR in alcohol-withdrawal symptoms (Sander *et al*, 1997; Schmidt *et al*, 1998; Gorwood *et al*, 2003). Its relevance to smoking behavior (Lerman *et al*, 1999; Sabol *et al*, 1999; Jorm *et al*, 2000; Vandenbergh *et al*, 2002; Erblich *et al*, 2005) is still tentative. With regard to the pathophysiology of stress-related disorders, the role of DAT VNTR in bipolar disorder is inconclusive as well (Souery *et al*, 1996; Gomez-Casero *et al*, 1996; Waldman *et al*, 1997; Georgieva *et al*, 2002). Neuroimaging studies observed a reduced striatal dopamine transporter binding in patients suffering from major depression (Meyer *et al*, 2001) or seasonal affective disorder (Neumeister *et al*, 2001), whereas another study reported elevated striatal dopamine transporter binding in depressed patients (Laasonen-Balk *et al*, 1999). In the light of the likely yet unclear influences of genetic variation in DAT on gene expression and transporter function, these divergent findings may not be surprising. It remains to be seen if the recent finding of an excess of the DAT 9-repeat allele in patients with PTSD (Segman *et al*, 2002) can be replicated more consistently.

The question arises how the interaction of BDNF and DAT on Neuroticism and Harm Avoidance may be explained on the basis of present knowledge. Indeed, there is strong evidence for various interactions of BDNF and the central dopaminergic system. Dopaminergic neurons in rat ventral midbrain were discovered to express BDNF-mRNA (Seroogy *et al*, 1994) and neurotrophin receptor TrkB and TrkC-mRNA (Numan and Seroogy, 1999). Several authors were able to detect BDNF in combination with dopamine to induce the dopaminergic phenotype in fetal rat and human cerebral cortex cultures (Hyman *et al*, 1994; Zhou *et al*, 1994, 1996, 1998; Theofilopoulos *et al*, 2001; Riaz *et al*, 2002, 2004). Stimulation with dopamine results in increased BDNF-mRNA and -protein in neuronal cultures (Küppers and Beyer, 2001). A BDNF infusion into the substantia nigra increases dopamine turnover in the striatum (Martin-Iverson *et al*, 1994), whereas a loss of BDNF expression leads both to downregulation of the dopaminergic phenotype and to dopaminergic neuronal death (Porritt *et al*, 2005). Recently, Berton *et al* (2006) reported that BDNF plays an essential role in the mesolimbic dopamine pathway in social defeat stress. Developing a murine model relevant to human psychiatric conditions such as depression, social phobia, and PTSD, mice showed a long-lasting social withdrawal after the experience of repeated aggression. Berton *et al* revealed that BDNF is required for the development of experience-dependent social aversion using a mesolimbic dopamine pathway-specific knockdown of BDNF. Local deletion of this neurotrophin in the ventral tegmental area—as well as chronic treatment with antidepressants—reverses the effect of social defeat. This profile is opposite to the antidepressant-like activity of BDNF reported in the above-mentioned hippocampal studies. In conclusion, there is a great amount of evidence that there is a close connection of central BDNF and the dopaminergic system.

Several studies revealed gene–gene interaction in the modification of personality dimensions. Noble *et al* (1998) found an association of the D2 and D4 receptors (DRD2, DRD4) and Novelty Seeking. The combined DRD2 and DRD4 polymorphisms contributed more markedly to this personality trait than when these two polymorphisms were considered individually. Furthermore, Benjamin *et al* (2000) described a replicable interaction of 5-HTT, DRD4, and COMT that influences Novelty Seeking scores (Strobel *et al*, 2003). It is likely that an interplay of several or multiple gene variants in particular determines a characteristic personality dimension.

In conclusion, our study reveals a DAT VNTR-dependent association between NEO-PI-R Neuroticism and the BDNF Val66Met polymorphism, but fails to replicate the previously reported direct association between the BDNF Met variant and low Neuroticism. Our results further support the notion that allelic variation at the BDNF locus—in interaction with several genes—influences anxiety- and depression-related personality traits and modulates liability to stress-related disorders.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)