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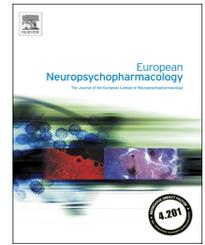
Impact of variation in the BDNF gene on social stress sensitivity and the buffering impact of positive emotions: Replication and extension of a gene–environment interaction

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Abstract
A previous study reported that social stress sensitivity is moderated by the brain-derived-neurotrophic-factor^{Val66Met} (BDNF rs6265) genotype. Additionally, positive emotions partially neutralize this moderating effect. The current study aimed to: (i) replicate in a new independent sample of subjects with residual depressive symptoms the moderating effect of BDNF^{Val66Met} genotype on social stress sensitivity, (ii) replicate the neutralizing impact of positive emotions, (iii) extend these analyses to other variations in the BDNF gene in the new independent sample and the original sample of non-depressed individuals.
Previous findings were replicated in an experience sampling method (ESM) study. Negative Affect (NA) responses to social stress were stronger in "Val/Met" carriers of BDNF^{Val66Met}

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compared to "Val/Val" carriers. Positive emotions neutralized the moderating effect of BDNF^{Val66Met} genotype on social stress sensitivity in a dose-response fashion. Finally, two of four additional BDNF SNPs (rs11030101, rs2049046) showed similar moderating effects on social stress-sensitivity across both samples. The neutralizing effect of positive emotions on the moderating effects of these two additional SNPs was found in one sample.

In conclusion, ESM has important advantages in gene-environment (GxE) research and may attribute to more consistent findings in future GxE research. This study shows how the impact of BDNF genetic variation on depressive symptoms may be explained by its impact on subtle daily life responses to social stress. Further, it shows that the generation of positive affect (PA) can buffer social stress sensitivity and partially undo the genetic susceptibility.

1. Introduction

In recent years, the study of gene-environment interactions (GxE) to unravel the pathogenesis of psychiatric disorders like major depressive disorder (MDD) has gained popularity (Caspi and Moffitt, 2006). The Brain-Derived Neurotrophic Factor (BDNF) gene has received much attention in recent GxE MDD research (Kaufman et al., 2006; Chen et al., 2012). The BDNF gene contains a functional polymorphism that results in a change from Valine (Val) in Methionine (Met) (BDNF^{Val66Met} genotype). BDNF is a protein encoded by the BDNF gene and supports survival and growth of neurons. BDNF^{Val66Met} is a common and naturally occurring variation in the BDNF gene and Met-carriers of this polymorphism have decreased secretion of BDNF. A higher number of Met-alleles are associated with a higher susceptibility for MDD and anxiety (Simons et al., 2009; Kalueff et al., 2006). In addition to the BDNF^{Val66Met} genotype, several other Single-Nucleotide Polymorphisms (SNPs) are probably associated with the diagnosis of MDD (Licinio et al., 2009).

Despite the growing interest in GxE interactions consistent replications of findings are rare (Moffitt et al., 2005; Duncan and Keller, 2011). Several explanations for these inconsistencies have been put forward. First, most initial GxE studies included measurements of *distal,* rather than *proximal,* environmental exposures, such as retrospective assessments of stressful events that occurred years ago (Caspi et al., 2003). These measurements may include error due to recall bias and mood-congruency effects at the moment when participants fill out questionnaires. In addition, the large time lag between exposure and the occurrence of variables of interest like a depressive episode allows for considerable noise generated by other factors that may have an impact on outcome. Thus, precision of environmental measurements used in GxE studies deserves more attention. Making use of more proximal, repetitive, and prospective environmental measurements increases precision (Moffitt et al., 2005; Zammit and Owen, 2006).

Second, the lack of consistency relates to the measurement of the outcome variable. Many GxE studies use a (dichotomous) psychiatric diagnosis as outcome variable. The use of these heterogeneous categories, characterized by disputable validity leads to high heterogeneity of outcome variables (Moffitt et al., 2005; Hasler and Northoff, 2011). An alternative strategy to examine etiological mechanisms that are involved in the development of psychiatric disorders is to focus directly on genes impacting on intermediate endophenotypes of psychiatric disorders. One of these putative intermediate endophenotypes in the etiology of MDD is increased stress sensitivity. Stress sensitivity is a dynamic phenotype that involves affective responses to small stressors in the flow of daily life (Csikszentmihalyi and Larson, 1987; Wichers et al., 2007a). A myriad of studies have reported that increased stress sensitivity is a risk factor for the development of psychiatric disorders such as MDD (Drabant et al., 2012; Wichers et al., 2009a) and psychosis (Mueller et al., 2011; Myin-Germeys et al., 2005a).

Third, Plues and Belsky (2012) recently argued that putative risk alleles often operate as 'plasticity' alleles, which is in line with the differential susceptibility hypothesis of Belsky (Belsky et al., 2009). According to Belsky's hypothesis people vary in 'developmental plasticity'. More "plastic or malleable" people are more vulnerable for adverse environmental influences but they may also be more impressionable for factors such as momentary positive affect resulting from positive events in their environment. On the other hand, less malleable people are less affected by environmental exposure. Plues and Belsky conclude that "the failure to explicitly measure and include positive supportive aspects of the environment in G × E studies may be an important reason why G × E findings fail to replicate (Pluess and Belsky, 2012, pg. 222)".

The experience sampling method (ESM) (Csikszentmihalyi and Larson, 1987), a self-assessment technique that is used to assess context, thoughts, affect, and symptoms in the flow of daily life, which can be used to investigate GxE in a momentary, prospective and 'real-world' design. Because environmental exposure and experience of stress are measured nearly simultaneously, measurement error due to recall bias and mood-congruency effects is minimized.

Some recent studies applied this methodology in examining the effect of genes on stress-sensitivity as a risk factor for MDD (Wichers et al., 2007a; Wichers et al., 2009a) and psychosis (Van Winkel et al., 2008; Simons et al., 2009; Collip et al., 2011). Only two ESM studies examined the moderating effect of the BDNF^{Val66Met} polymorphism on stress sensitivity, operationalized as emotional responses to minor stressors in daily life (Simons et al., 2009; Wichers et al., 2008b). These studies reported that Met-carriers respond with more negative affect (NA) or paranoia to minor daily life stressors.

Additionally, two studies (Wichers et al., 2008b; Wichers et al., 2007b) showed that the ability to experience positive emotions during daily life stressors neutralized in part the

genetic impact on stress-sensitivity. Individuals with the BDNF 'Met' risk allele showed higher levels of stress sensitivity, in a context of low positive emotions, than individuals with the 'Val' allele. But when they experienced high levels of positive emotions, 'Met' carriers were able to buffer their increased social stress sensitivity and showed similar levels of stress sensitivity as 'Val' carriers. New studies are necessary, however, to examine whether these results can be replicated and whether similar effects can be found for other genetic variations in the BDNF gene, implicated in MDD.

The aim of this study is threefold: to replicate in a sample of subjects with residual depressive symptoms previous findings (Wichers et al., 2008b) into (i) the effects of the BDNF^{Val66Met} polymorphism on stress responses to social stressors using ESM, (ii) the effect of positive emotions on this GxE result, and (iii) to extend the analyses with four additional SNPs of the BDNF gene, which are not in linkage disequilibrium with the BDNF^{Val66Met} genotype, in the original twin sample and the new sample of subjects with residual depressive symptoms.

2. Experimental procedures

2.1. Subjects

Sample 1: A sample of 130 individuals with residual depressive symptomatology after at least one episode of MDD were recruited from outpatient healthcare facilities and through posters in public areas in Maastricht (Geschwind et al., 2011). Residual symptoms were defined as a score of seven or higher on the 17-item Hamilton Depression Rating Scale (HDRS; Hamilton, 1960) at the time of screening. Exclusion criteria were fulfilling criteria for a current major depressive episode, schizophrenia, psychotic episodes in the past year, and recent (past four weeks) or upcoming changes in ongoing psychological or pharmacological treatment.

An initial screening of potential participants was performed by phone to check for availability during the study period and likelihood of meeting in- and exclusion criteria. A second screening included administration of the Structured Clinical Interview for DSM IV axis I (SCID-I; First et al., 1995) by trained psychologists and the 17-item HDRS by trained research assistants. The project was approved by the Local Ethics Committee. Demographic variables are shown in Table 1 under subject characteristics.

Sample 2: The second sample consisted of 621 female twins recruited from an ongoing longitudinal, general population twin study, named the East-Flanders Prospective Twin Survey. This population-based survey has prospectively recorded all multiple births in the province of East Flanders since 1964 (Derom et al., 2013). Subjects were Caucasian and of Belgian origin. The project was approved by the Local Ethics committee. For more details on the sample see Wichers et al. (2008b). Demographic variables are shown in Table 1 under subject characteristics.

2.2. Experience sampling method

The experience sampling method (ESM) is a momentary assessment method to assess participants in their daily living environment, providing repeated in-the-moment assessments of affect in a prospective and ecologically valid manner (Delespaul, 1995). Participants in both samples received a digital wristwatch and a set of ESM self-assessment forms collated in a booklet for each day. The wristwatch was programmed to emit a signal ("beep") at an unpredictable moment in each of ten 90-min time blocks between

Table 1 Demographics and characteristics of sample 1 and 2.

Variable	Sample 1 (n=127)	Sample 2 (n=446)
Mean age (years)	44	28
Gender (Female) %	76	100
Fulltime / parttime work %	65	60
Illness / unemployment benefits %	21	3
Living with partner / own family %	64	94
Current episode of MDD %	0	4.9
Current use of antidepressants %	35	2.7
Mean positive affect (PA) (S.D.)	2.18 (1.25)	4.44 (0.83)
Mean negative affect (NA) (S.D.)	1.96 (1.06)	1.26 (0.35)
Mean social stress (S.D.)	2.20 (1.29)	2.61 (1.47)

7:30 and 22:30, on five to six consecutive days, resulting in a maximum of 60 beeps per person. After each beep, participants were asked to fill out the ESM self-assessment, collecting reports of current mood and context. All self-assessments were rated on 7-point Likert scales. Participants were instructed to complete their reports immediately after the beep, thus minimizing memory distortion, and to record the time at which they completed the form. Participants with less than one third of valid reports were excluded from the analysis, and all reports not completed within 15 min after the actual beep were considered invalid, as previous work (Csikszentmihalyi and Larson, 1987; Delespaul, 1995) has shown that reports completed after this interval are less reliable and consequently less valid.

2.3. Measurements

2.3.1. Social stress
Social stress in both samples was measured by asking subjects whether they were alone at the time of the beep. If they were not alone, they were asked how much they liked the company they were in at the time of the beep. Subjects rated their company on a 7-point likert scale ranging from 1 (not at all) to 7 (very much). The scale was reversed so that higher scores represent higher disliking of being in that company (social stress).

2.3.2. Momentary affective states
At each beep, several ESM mood adjectives were assessed on 7-point Likert scales ranging from 1 (not at all) to 7 (very). Consistent with previous work (Wichers et al., 2010; Myin-Germeys et al., 2001), principal component factor analysis with oblique rotation was used to generate a factor representing PA and a factor representing NA.

There was a slight difference between the two samples concerning the used items. In sample 1 the mood adjectives "cheerful", "content", "strong," and "enthusiastic" loaded on the PA factor and the respective loadings were 0.86, 0.84, 0.81, and 0.90. For analyses of sample 2 "strong" was replaced by "energetic", since the adjective "strong" had not been measured in this sample. The mood adjectives "cheerful," "content," "energetic," and "enthusiastic" formed positive affect (PA). Respective loadings were 0.86,

0.74, 0.84, and 0.88. The mean of these four items formed the PA scale in both samples.

The mood items "insecure," "lonely," "anxious," "down," "guilty," and "suspicious" were used to assess negative affect (NA) in both samples. Respective loadings were 0.74, 0.66, 0.80, 0.54, 0.71, 0.80 for sample 1 and 0.77, 0.58, 0.73, 0.65, 0.62, 0.68 for sample 2. The mean of these six items formed the NA scale in both samples. PA and NA were computed for each beep moment.

2.4. Genotyping

Genomic DNA was obtained in two stages. In the first stage the BDNF^{Val66Met} genotype was genotyped for sample 2. Out of 621 subjects, 129 refused genotyping, and for the 492 consenting subjects, samples suitable for DNA analysis were available for 488 subjects. Placental tissue for DNA analysis was available for 156 subjects, blood samples for 14, and buccal cell samples for 208, using a sterile swab specifically designed for the collection of buccal cell samples for DNA testing (Omni Swabs; Whatman plc, Brentford, England). Genomic DNA was extracted using QIAamp DNA Mini Kits (Qiagen, Venlo, the Netherlands), according to the appropriate protocol for each sample type. BDNF^{Val66Met} genotype was determined by KBioscience (Hertz, UK) using their proprietary allelic discrimination assay. For every monozygotic (MZ) twin in the sample with genotypic data, the same genotypic data were included for the co-twin, assuming that both twins had identical genotypes.

In the second stage the BDNF^{Val66Met} SNP was genotyped in sample 1 and several (additional) BDNF SNPs were targeted for both samples based on two relevant reports at that time. The choices for these additional SNPs were made based on recent papers showing that these particular BDNF genotypes were associated with MDD (Licinio et al., 2009): rs56820186, rs6265, rs11030101, rs28722151, rs11030102, rs57083135, rs110303103, rs12273539 and other kinds of psychopathology, like schizophrenia (Nicodemus et al., 2008): rs56164415 and rs2049046.

For sample 2, the DNA of 400 subjects was available for analysis of additional BDNF SNPs. In sample 1 genomic DNA was available for 127 subjects. DNA was obtained from saliva samples. Saliva was collected in Oragene-DNA Self Collection Kits (DNA Genotek, Ottawa, Canada), and DNA was isolated using the AutoGenFlex DNA isolation system (Autgen, Hilliston, MA, USA) according to manufacturer's instructions. SNPs within the BDNF gene were determined by Sequenom (Hamburg, Germany) using the Sequenom MassARRAY iPLEX platform at the facilities of the manufacturer.

Some of the above BDNF polymorphisms were not available for analysis:

1. SNPs rs56820186, and rs11030103 were not determined due to methodological limitations of the genotyping procedure.
2. SNP rs28722151 was marked as 'suspect' in dbSNP (Sherry et al., 2001), indicating probable sequencing error rather than representing a real polymorphic locus. Therefore, we did not include this SNP in our study.
3. SNP rs56164415 was not in Hardy-Weinberg equilibrium for sample 2 and therefore not analyzed.
4. SNPs rs12273539 and rs57083135 were not analyzed because the frequencies of the minor allele were too low ($<$10%) to allow for meaningful analysis.

In total, the genotyping procedure lead to four analyzable additional BDNF SNPs: rs11030101, rs2049046, rs56164415 (only sample 1), and rs11030102.

2.5. Analyses

ESM data have a hierarchical structure. This means that multiple observations (Level 1) were clustered within subjects (Level 2) and multiple subjects within twin pairs (level 3; only in sample 2). A multilevel analysis takes the variability associated with each level into account (Snijders and Bosker, 1999). The XTMIXED command in STATA 12.1 (StataCorp, 2011) was applied to the data. First, we examined the moderating effect of the BDNF^{Val66Met} genotype on the NA response to daily social stress in sample 1. Additionally, we investigated the three-way interaction between momentary PA, BDNF^{Val66Met} genotype and daily social stress on NA response in sample 1 to examine the extent to which PA reduces the moderating effect of the BDNF^{Val66Met} genotype. Dose-response associations were examined by dividing levels of momentary experiences of PA in three tertiles: low PA (33% with lowest PA score), average PA (33% middle group) and high PA (33% with highest PA score). Third, these analyses were repeated for additional BDNF SNPs in both samples. In order to ensure that significant findings are not due to chance we used the following strategy: we performed our analyses in two independent samples. If findings are replicated in another independent sample, with a p-value of below 0.05 for both samples, results are most likely not due to chance (see for example: Williams and Haines, 2011).

3. Results

3.1. Subject characteristics

Sample 1: Out of 130 participants, 127 subjects yielded valid BDNF^{Val66Met} genotype measurements and ESM measurements with respect to social stress. Due to the repeated measurements within the ESM framework this resulted in 3417 observations. The sample size for additional BDNF SNPs was smaller due to a few invalid genotype measurements. For SNP rs11030101, rs11030102 and rs2049046 this resulted in a sample size of 124 subjects with 3347 observations. The sample size for rs56164415 consisted of 123 subjects with 3321 observations.

Sample 2: Out of the 492 consenting subjects, 480 yielded valid BDNF^{Val66Met} genotype measurements (including genotype derived from MZ twin sister). Of the 480 subjects, seven were not included due to ambiguity concerning the type of zygosity. Another 27 had incomplete ESM measurements with respect to social stress. This resulted in a sample of 446 subjects with 15,863 observations for the BDNF^{Val66Met} genotype. Of the 446 subjects, 420 were female members of twin pairs (268 subjects were members of MZ twin pairs, 150 subjects were members of dizygotic (DZ) twin pairs, one pair was of unknown zygosity), and 26 were non-twin sisters.

For the additional BDNF SNPs, valid genotype measurements were available for 396 subjects (including genotype derived from MZ twin sister) for SNP rs11030102 and for 397 subjects for SNPs rs11030101 and rs2049046 (out of the original 400 subjects). 22 Subjects had incomplete ESM measurements with respect to social stress. This resulted in a sample size of 375 subjects for SNPs rs11030101 and rs2049046 with 13249 observations and for rs11030102 in a sample size of 374 subjects with 13208 observations.

In both samples the frequencies of the Met/Met variant of the BDNF^{Val66Met} genotype were very small, which is commensurate with previous literature (Lang et al., 2005; Schule et al., 2006). The frequencies of the G/G variant of the BDNF SNP

rs11030102 were also very small. For the specific frequencies of the various SNPs that are taken into analyses, we refer to Table 2. We therefore decided to not perform statistical analyses on these genotypes. SNP rs11030101 and rs2049046 are in almost perfect linkage disequilibrium. Correlations among other SNPs were not higher than −0.561. For the specific correlations between the various SNPs that are taken into analyses, we refer to Table 3.

3.2. Effects of the BDNF^{Val66Met} genotype on NA response to social stress (sample 1)

In the analyses the Val/Val variant was used as reference group. Val/Met subjects showed increased NA responses in comparison with Val/Val subjects when they were experiencing social stress ($B=0.063$, $p=0.008$). Fig. 1 shows the increase in NA stratified by BDNF genotype (Val/Val; Val/Met) and level of appraisal of company. Differences in effect sizes between the Val/Val and Val/Met variants were greatest for below average pleasant

Table 2 Frequencies (%) of the allelic variants of the BDNF SNPs taken into analysis.

BDNF SNP	Allelic variants	Sample 1 (%)	Sample 2 (%)
BDNF ^{Val66Met} (rs6265)	Val/Val	62.9	65.5
	Val/Met	33.1	29.6
	Met/Met	4.1	4.9
rs11030102	C/C	58,7	53.1
	C/G	35.2	40.8
	G/G	6.1	6.1
rs11030101	A/A	28.9	30.0
	A/T	49,3	50.2
	T/T	21.7	19.8
rs56164415	G/G	87.7	–
	A/G	12.3	–
rs2049046	A/A	28.9	29.2
	A/T	49.3	50.4
	T/T	21.7	20.2

($B=0.458$, $p=0.058$) and very unpleasant appraisals ($B=0.664$, $p=0.006$) of company.

For the results of the moderating effect of BDNF^{Val66Met} genotype on NA to social stress in the original twin sample (sample 2 in this paper) we refer to a previous publication (Wichers et al., 2008b).

3.3. The moderation of the BDNF ^{Val66Met} genotype x social stress interaction by PA (sample 1)

Results showed that the moderating effect of PA on NA response to social stress was significantly stronger in BDNF Val/Met than Val/Val carriers ($B=-0.040$, $p=0.04$). A dose-response association was apparent only in BDNF Val/Met participants such that higher levels of PA were associated with lower NA responses to social stress (see Fig. 2). Effect sizes of BDNF Val/Val and Val/Met stratified for low, average and high PA are shown in Fig. 2. Also here, we refer to a previous publication for the results on the same analysis in sample 2 (Wichers et al., 2008b).

3.4. Extension: effects of the additional BDNF SNPs on NA response to social stress (sample 1 and 2)

The following results refer to analyses on the additional SNPs rs11030101, rs2049046, rs56164415, rs11030102. These analyses were performed on both samples, except for SNP rs56164415 that was only taken into analysis for sample 1. Effect sizes for the interaction effect between BDNF SNPs and social stress on NA are shown in Table 4.

For two SNPs interaction effects were significant in both samples, but the overall increase in NA in response to social stress is lower in the sample of a general population (sample 2) than in the sample of people with residual depressive symptoms (sample 1). In comparison with the reference group A/A, subjects with the A/T variant of SNP rs11030101 showed a significant higher increase of NA in response to social stress in both samples (sample 1: $B=0.075$, $p=0.003$; sample 2: $B=0.016$, $p=0.001$). The results for subjects with the A/T variant in comparison with the reference group are graphically shown in Fig. 3a and b for both samples. Differences in effect sizes between subjects with the A/T variant of SNP rs11030101 and the

**Table 3** Correlates of the allelic variants of the BDNF SNPs taken into analysis (Sample 1 and 2).

BDNF SNP	BDNF ^{Val66Met} (rs6265)	rs11030102	rs11030101	rs56164415	rs2049046
BDNF ^{Val66Met} (rs6265)	1.00				
rs11030102					
Sample 1:	−0.079	1.00			
Sample 2:	−0.359				
rs11030101					
Sample 1:	−0.437	−0.561	1.00		
Sample 2:	−0.424	−0.521			
rs56164415					
Sample 1:	−0.184	−0.079	−0.249	1.00	
rs2049046					
Sample 1:	−0.437	−0.561	1.00	−0.249	1.00
Sample 2:	−0.396	−0.521	0.980	/	
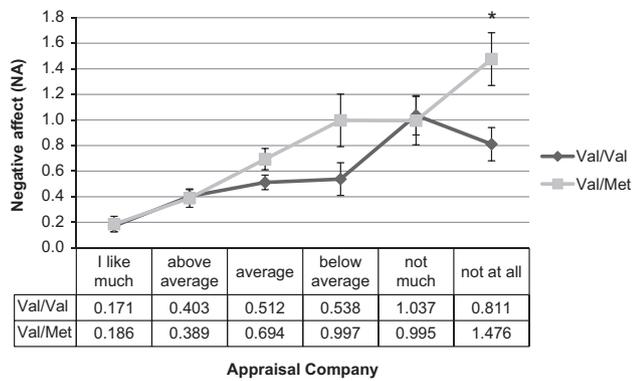
Fig. 1 Effect of the BDNF[Val66Met] genotype (rs6265) on negative affect (NA) responses to social stress (Sample 1). Single asterisk represents significant difference in effect sizes for very unpleasant appraisals of company (effect of social stress on negative affect) between the two BDNF[Val66Met] genotype variants. Val/Val = Valine/Valine variant; Val/Met = Valine/Methionin variant.
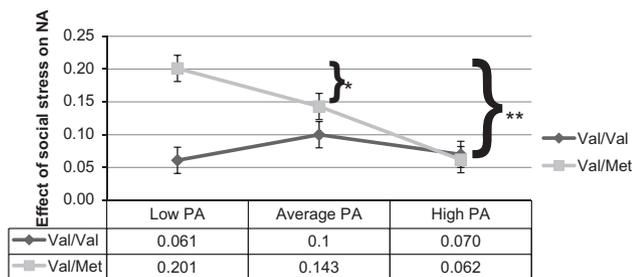
Fig. 2 Positive affect (PA) moderating interaction effect of BDNF[Val66Met] genotype x social stress on negative affect (NA). Single asterisk represents significant decrease in effect sizes from Low to Average PA ($B = -0.103$, $p = 0.024$) on NA responses to social stress of the BDNF[Val66Met] genotype. Double asterisk represents significant decrease in effect sizes from Low to High PA ($B = -0.154$, $p = 0.003$) on NA responses to social stress of the BDNF[Val66Met] genotype. The BDNF[Val66Val] genotype shows no significant decrease in effect size on NA responses to social stress as experience of PA increases.

reference group were greatest for very unpleasant appraisals (sample 1: $B = 0.874$, $p = 0.001$; sample 2: $B = 0.119$, $p = 0.003$) of company in both samples. Subjects with the T/T variant showed, compared to the reference group, a significant increased NA response to social stress in sample 2 ($B = 0.013$, $p = 0.025$ but not in sample 1 ($B = 0.019$, $p = 0.543$).

Subjects with the A/T variant of SNP rs2049046 showed also a significant higher beep level NA response in the context of social stress in both samples (sample 1: $B = 0.075$, $p = 0.003$; sample 2: $B = 0.015$, $p = 0.001$). Subjects with the T/T variant showed in comparison with the reference group only in sample 2 a significant increased NA response when confronted with social stress ($B = 0.023$, $p < 0.001$). The results for this SNP are not graphically shown because this polymorphism is in almost perfect linkage disequilibrium with SNP rs11030101.

3.5. Extension: the moderation of additional BDNF SNPs x social stress interaction by PA (sample 1 and 2)

For both SNP's rs11030101 A/T and rs2049046 A/T, the negatively moderating effect of PA on the impact of the BDNF SNPs on NA response to social stress was only present in sample 1. A dose–response association was apparent in that higher levels of PA were associated with less impact of variation in the above two SNPs on NA responses to social stress. This was found only in sample 1 ($B = -0.057$, $p = 0.004$) but not in sample 2 for rs11030101 ($B = -0.003$, $p = 0.459$) and rs2049046 ($B = 0.004$, $p = 0.353$).

Further, a negatively moderating effect of PA on the impact of the BDNF SNPs on NA response to social stress was apparent in sample 2 for the T/T variant of rs2049046 ($B = -0.021$, $p < 0.001$) and the C/G variant of rs11030102 ($B = 0.011$, $p < 0.001$), but not in sample 1.

4. Discussion

The current paper replicated an interaction effect between the BDNF[Val66Met] genotype and momentary social stress on momentary negative affect in daily life. We also replicated the neutralizing impact of PA on the contribution of variation in the BDNF[Val66Met] genotype to social stress-sensitivity. Moreover, similar effects were found for additional SNPs of the BDNF gene, some of which were replicated in two independent samples.

4.1. Comparison to previous literature

These findings are in line with other GxE studies suggesting that variations in the BDNF genotype are associated with MDD (Carver et al., 2011; Perea et al., 2012; Wong et al., 2012). However, two recent meta-analyses show inconclusive or even negative results (Chen et al., 2008; Lopez-leon et al., 2008), suggesting there is no association between the BDNF[Val66Met] genotype and MDD. One meta-analysis showed an association between MDD and the BDNF[Val66Met] genotype for males (Verhagen et al., 2010).

As outlined in the introduction, methodological limitations may be responsible for inconsistencies in the literature. ESM may provide a methodological approach to overcome these limitations. The results of our study show that with the use of ESM methodology, we were able to replicate effects of BDNF SNPs on social stress sensitivity. ESM measures environmental exposure in a more proximal, repetitively, and prospective way, and creates the possibility to measure possible protective environmental aspects.

Moreover, ESM is complementary to epidemiological studies. Whereas these studies help in finding out *that* BDNF is associated with depression (Licinio et al., 2009; Wong et al., 2012), ESM allows us to zoom in on the potential underlying mechanisms behind the association and helps to increase insights in *why* variation in the BDNF gene may be associated with MDD (Wichers, 2013). If individuals respond to everyday occurring social stressors with a little more NA multiple times a day then, cumulatively, this may have a large impact on the individual's wellbeing. Logically it follows that genetic variants associated with altered NA

Table 4 Effect sizes for the interaction effect between the additional BDNF SNPs and social stress on momentary negative affect (NA) (two-way interaction) and the moderating effect of positive affect (PA) on the BDNF SNP x social stress interaction (three-way interaction).

BDNF SNP	rs11030101 A/T	rs11030101 T/T	rs2049046 A/T	rs2049046 T/T	rs56164415 A/G	rs11030102 C/G
Two-way interaction SNP & social stress on NA in sample 1	$B=0.075$, $p=0.003$	$B=0.019$, $p=0.543$	$B=0.075$, $p=0.003$	$B=0.019$, $p=0.543$	$B=-0.068$, $p=0.019$	$B=-0.001$, $p=0.702$
Two-way interaction SNP & social stress on NA in sample 2	$B=0.016$, $p=0.001$	$B=0.013$, $p=0.025$	$B=0.010$, $p=0.027$	$B=0.023$, $p<0.001$	–	$B=-0.010$, $p=0.010$
Three-way interaction SNP & social stress & PA in sample 1	$B=-0.057$, $p=0.004$	$B=0.009$, $p=0.694$	$B=-0.057$, $p=0.004$	$B=0.008$, $p=0.694$	$B=0.028$, $p=0.238$	$B=-0.013$, $p=0.486$
Three-way interaction SNP & social stress & PA in sample 2	$B=-0.003$, $p=0.459$	$B=-0.007$, $p=0.129$	$B=0.004$, $p=0.353$	$B=-0.021$, $p<0.001$	–	$B=0.011$, $p<0.001$

The above variants of the various BDNF genotypes are compared with their reference group.
The reference group for SNP rs11030101 is the A/A variant.
The reference group for SNP rs2049046 is the A/A variant.
The reference group for SNP rs56164415 is the G/G variant.
The reference group for SNP rs11030102 is the C/C variant.

responses to everyday stress may put individuals at increased risk for MDD (Wichers et al., 2009a; Wichers et al., 2008a). This is in line with other research that has put social stress and individual stress sensitivity forward as a possible underlying mechanism in the development of MDD (Kaufman et al., 2006; Morris et al., 2012). A deeper understanding of the way in which genes impact on our behavior and experience is relevant to understand in what way genetic risk can be countered. In this respect, research at the micro-level of experience may complement macro-scale epidemiological research.

4.2. Moderation of the interaction effects by positive emotions

In the current study, we replicated the neutralizing effect of PA on the genetic impact of the BDNF[Val66Met] genotype on social stress sensitivity that was reported in previous ESM studies (Wichers et al., 2007b, 2008b). Higher levels of PA buffer the genetic susceptibility for social stress in BDNF Val/Met carriers, resulting in similar NA levels in comparison with Val/Val carriers. These findings are in accordance with the hypothesis that some genetic variants make people differ in their sensitivity to context (Ellis and Boyce, 2011) and support the differential susceptibility hypothesis of Belsky (Belsky et al., 2009). Val/Met carriers of the BDNF[Val66Met] genotype showed increased sensitivity to negative social contexts but they also seem to profit more from momentary positive affect. On the contrary, Val/Val carriers are less susceptible to social stress, but are also less affected by positive affect.

For rs11030101 A/T and rs2049046 A/T we found a buffering role for positive emotions in male and female subjects with residual depressive symptoms (sample 1), but not in healthy female twins (sample 2). Several reasons may explain this finding. First, a recent meta-analysis showed significant effects of BDNF[Val66Met] genotype on MDD for men but not for women (Verhagen et al., 2010). We tested post-hoc if the difference between our samples could be accounted for by gender. This appeared not to be the case,
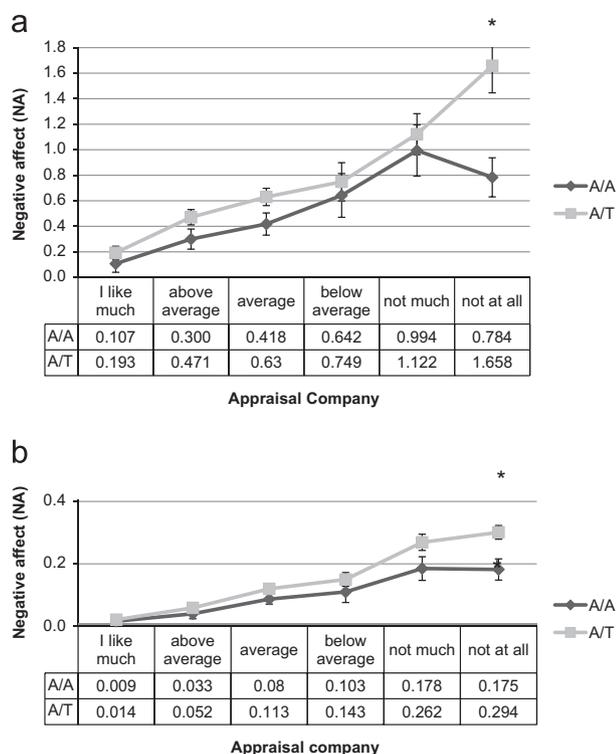
as the results for sample 1 remained the same when males were excluded. Second, the reason that we found no buffering role for PA in healthy female twins (sample 2) could be the result of a floor effect of social stress sensitivity in healthy individuals, which may lead to a lower power level in comparison with sample 1, which contains individuals with residual depressive symptoms. Third, we cannot exclude the possibility that these findings are a result of a type I-error. In future research, replication of these findings is necessary.

The replicated finding that PA can neutralize genetic risk has important clinical implications. Other reports have already highlighted the importance of efficient generation of PA as a hallmark in not only maintaining and restoring a healthy emotional balance, but also in the prevention of and recovery from MDD (Garland et al., 2010; Wichers et al., 2009b; Geschwind et al., 2011). Therefore, therapeutic strategies that specifically aim at increasing the experience of PA in daily life in subjects vulnerable to or suffering from low mood may have high clinical utility. In conclusion, the neutralizing effect of PA suggests that a non-deterministic view on the mechanisms of genes on our experience and behavior is preferable.

4.3. Methodological issues

Few males were included in sample 1 ($n=30$) and none in sample 2. Therefore, our findings cannot be extrapolated to the male population; the influence of gender will have to be addressed in future studies. Additionally, few subjects displayed the Met/Met variant of the BDNF [Val66Met] genotype and the G/G variant of the BDNF SNP rs11030102. This current study is not informative with respect to these variants.

The BDNF [Val66Met] genotype is known as a functional polymorphism. At this moment it remains unclear to what level rs11030101 and rs2049046 have an (functional) influence on BDNF protein expression or function. SNPs rs11030101 and rs2049046 are statistically independent of the BDNF [Val66Met] genotype, suggesting that (one of) these SNPs may have

	I like much	above average	average	below average	not much	not at all
A/A	0.107	0.300	0.418	0.642	0.994	0.784
A/T	0.193	0.471	0.63	0.749	1.122	1.658

	I like much	above average	average	below average	not much	not at all
A/A	0.009	0.033	0.08	0.103	0.178	0.175
A/T	0.014	0.052	0.113	0.143	0.262	0.294

Fig. 3 (a) BDNF gene rs11030101 moderating negative affect (NA) in response to social stress (sample 1). Single asterisk represents significant difference in unstandardized effect sizes for very unpleasant appraisals of company (effect of social stress on negative affect) between the two genotype variants, A/A and A/T. (b) BDNF gene rs11030101 moderating negative affect (NA) in response to social stress (sample 2). Single asterisk represents significant difference in effect sizes for very unpleasant appraisals of company (effect of social stress on negative affect) between the two genotype variants, A/A and A/T.

functional properties or be in linkage with an as yet unidentified functional variant.

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Contributors

Author M. Wichers designed the study and wrote the protocol. Author M. van Winkel managed the literature searches and analyses, the statistical analysis, and wrote the first draft of the manuscript. Authors M. Wichers and F. Peeters contributed by correcting the draft during the writing stage. All authors contributed to and have approved the final manuscript.

Conflict of interest

There is no conflict of interest.

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