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BDNF Val^{66}Met polymorphism is associated with higher anticipatory cortisol stress response, anxiety, and alcohol consumption in healthy adults

Lorenza S. Colzato [a,*], A.J. Willem Van der Does [b], Coen Kouwenhoven [a], Bernet M. Elzinga [b], Bernhard Hommel [a]

[a] *Leiden University, Institute of Psychology, Department of Psychology, Cognitive Psychology Unit, Postbus 9555, 2300 RB Leiden, The Netherlands*
[b] *Leiden University, Clinical Psychology Unit and Leiden Institute for Brain and Cognition, Leiden, The Netherlands*

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Summary
Background: The brain-derived neurotrophic factor (BDNF) is a key protein in maintaining neuronal integrity. The BDNF gene is thought to play an important role in the pathophysiology of mood and anxiety disorders. The aim of this study was to investigate, for the first time in a single study, the association between BDNF Val^{66}Met polymorphism, anxiety, alcohol consumption, and cortisol stress response.
Method: 98 healthy university students (54 females and 44 males), genotyped for the Val^{66}Met polymorphism, participated in a physical-stress procedure (cold pressure test, CPT) after having been informed that they would undergo a painful experience. Indices of anxiety and of stress were collected from repeated measurement of salivary cortisol, blood pressure, and heart rate.
Results: BDNF Met carriers, were more anxious during the CPT ($p < 0.001$), drank more alcohol per week, ($p < 0.05$), and showed significantly higher anticipatory cortisol response ($p < 0.05$), but not in response to the CPT, than Val/Val homozygotes. The association of BDNF Val^{66}Met polymorphism with HPA axis reactivity to stress was not modulated by gender. These results suggest that Met carriers are particularly sensitive in anticipating stressful events, which extends previous findings on the moderating role of the BDNF Val^{66}Met polymorphism in the face of stressful life events.

1. Introduction

Cortisol is traditionally viewed as the most important stress hormone in humans (Sapolsky et al., 2000). In recent years cortisol has been shown to play a much broader role in human

* Corresponding author. Tel.: +31 071 5273407; fax: +31 071 5273783.
E-mail address: colzato@fsw.leidenuniv.nl (L.S. Colzato).

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functioning (Erickson et al., 2003). Several authors have attributed various problems in health and wellbeing to the impaired attunement of individuals to their environment, as a consequence of a dysfunctional hypothalamic–pituitary–adrenal (HPA) axis – the major pathway for regulating stress responses – and its cortisol production (e.g. McEwen, 1998b; Corbett et al., 2009).

A neurotrophin that has been shown to regulate the HPA response to stress is the brain-derived neurotrophic factor (BDNF). BDNF is a critical regulator of the formation, plasticity, and integrity of neurons in brain circuits that regulate emotion (Angelucci et al., 2005; Duman and Monteggia, 2006). In animals, exposure to stress early in life (for example, repeated maternal separation) has been found to induce a relative decrease in the expression of BDNF and to subsequent neuronal atrophy and degeneration in the hippocampus and the cortex, which can persist into adulthood (Murakami et al., 2005; Roceri et al., 2004; Song et al., 2006). Duman and Monteggia (2006) suggested that decreased expression of BDNF contributes to depression and that upregulation of BDNF plays a role in the actions of antidepressant treatment. Along the same line, Sertoz and colleagues (2008) suggest that low BDNF might be associated with burnout symptoms including altered mood and cognitive functions. The interaction between BDNF and HPA is even more complicated by evidence suggesting that neuroactive steroids, as dehydroepiandrosterone (DHEA), pregnenolone (PREG), modulate both HPA axis and BDNF protein levels (Naert et al., 2007). Moreover, it seems that in men plasma BDNF levels, as well as cortisol levels, are significantly higher in the morning than in the night (Begliuomini et al., 2008).

A promising functional single nucleotide polymorphism (SNP) that leads to an exchange of amino acids from valine (Val) to methionine (Met) has been found at codon 66 in the 5′ pro domain of the BDNF gene (Egan et al., 2003). The Met allele is associated with a decrease in activity-dependent secretion of BDNF compared to the Val allele (Egan et al., 2003). Given that the Met/Met homozygote variant occurs in only 2–3% of the Caucasian population, most studies have compared carriers of a Met allele (Val/Met) with individuals who are homozygous for the Val allele (Val/Val). Interestingly, Shalev et al. (2009) showed that BDNF Val^{66}Met is associated with HPA axis reactivity to psychological stress: male Val/Val homozygotes showed a greater rise in salivary cortisol than Val/Met heterozygotes, while female participants exhibited a trend towards the opposite response. Along the same line, Schüle et al. (2006) showed, by means of the fluorescence resonance energy transfer method (FRET), that Met carriers were associated with a significantly higher HPA axis activity during the dexamethasone/CRH test than Val/Val homozygotes.

Vinberg et al. (2009) demonstrated that individuals at high risk of affective disorders and who are Met carriers may exhibit an enhanced stress response. However, the altered stress response may be expressed only in combination with other risk variants through interactions with the environment. Furthermore, Elzinga et al. (2011) showed that BDNF Val^{66}Met moderated the effects of childhood abuse and recent stress on BDNF levels, with the Met carriers with a history of childhood abuse (CA) having lower BDNF levels compared to Met carriers without a history of CA, whereas this pattern was reversed in the Val/Val group. Moreover, Met carriers have also been found to be more anxious and depressed, both in animal studies (Chen et al., 2006) and in human studies (Jiang et al., 2005; Frodl et al., 2008; Gatt et al., 2009; Montag et al., 2009; Montag et al., 2010; Verhagen et al., 2010). In contrast to all these studies, Lang et al. (2005) found higher anxiety scores, as measured by the State Trait Anxiety Inventory (STAI), which allows anxiety to be quantified as a comparatively stable personality trait, in Val/Val homozygous individuals compared to Met carriers.

Besides being involved in regulating the HPA axis, the BDNF Val^{66}Met polymorphism seems to be involved in alcohol addiction, craving, and withdrawal (for a review, see Davis, 2008). First, the Met/Met BDNF polymorphism has been associated with alcoholism in violent alcoholics (Matsushita et al., 2004). Second, animal and in vitro studies suggest that short-term exposure to alcohol may increase BDNF levels in an effort to maintain homeostasis, but that long-term alcohol use causes both a decrease in BDNF levels in the hippocampus and hippocampal atrophy. In humans, Korean alcoholic patients were shown to have lower plasma BDNF levels (Joe et al., 2007). All these studies suggest that both low BDNF levels and the Met allele are associated with long-term alcohol use.

Interestingly, the number of alcohol units consumed per week and heavy drinking are positively associated with plasma cortisol levels (Gianoulakis et al., 2003) and salivary cortisol levels (Badrick et al., 2010). Dai et al. (2002) found that a single drink in advance of a stressor prevented the cortisol response to that stressor.

The purpose of the present study was to bring the pieces of this puzzle together in order to understand the multifaceted relation between BDNF Val^{66}Met polymorphism, HPA axis reactivity, anxiety, and alcohol consumption. Furthermore, we aimed at extending previous findings of Shalev et al. (2009) and investigate whether physical stress may also induce higher cortisol stress response in association with the BDNF Val^{66}Met polymorphism. In order to do that, healthy university students, genotyped for the Val^{66}Met polymorphism, we asked to participate in a physical-stress procedure (cold pressure test: CPT) and informed beforehand that this would be a painful experience. Indices of anxiety and of stress were collected from repeated measurement of salivary cortisol, blood pressure, and heart rate. To test whether anxiety and/or general neurotic trait may be correlated with alcohol consumption in Met carriers, participants filled out a personality trait questionnaire. We expected Met carriers to show higher cortisol levels in response to the cold pressure test, to be more anxious, and to consume more alcohol than Val/Val homozygotes.

2. Materials and methods

2.1. Participants

98 young Caucasian healthy adults (44 male/54 female), with a mean age of 22.2 years (SD = 2.6, range 18–30), served as participants for partial fulfilment of course credit or a financial reward. The sample was drawn from 110 adults in the Leiden and Rotterdam Metropolitan area (The Netherlands), who volunteered to participate in studies of behavioral genetics. Participants were recruited via ads posted on

community bulletin boards and by word of mouth. Exclusion criteria were any major medical illness that could affect brain function, current and/or past substance abuse, neurological conditions, endocrine illness, history of head injury, and history of psychiatric medical treatment. Participants were selected individually via a phone interview by the same lab-assistant using the Mini International Neuropsychiatric Interview (M.I.N.I.; Sheehan et al., 1998). The M.I.N.I. is a well established brief diagnostic tool in clinical and stress research that screens for several psychiatric disorders including schizophrenia, depression, mania, ADHD, and obsessive-compulsive disorder (Sheehan et al., 1998; Elzinga et al., 2007; Elzinga et al., 2008). Based on the interview, we excluded 12 of the 110 potential participants because of hints to a possible psychiatric disorder (ADHD, Mania) and/or substance abuse (cannabis).

Written informed consent was obtained from all participants after the nature of the study was explained to them; the protocol was approved by the institutional review board (Leiden University, Institute for Psychological Research).

2.2. Questionnaires

Participants filled out the Liebowitz Social Anxiety Scale (LSAS), Eysenck's personality questionnaire (EPQ-RSS), and one rating scale about weekly alcohol consumption. The LSAS (Liebowitz, 1987) consists of 24 short descriptions of social situations and participants have to indicate to what degree (none, mild, moderate, severe) those situations cause them anxiety or fear. The EPQ-RSS questionnaire consists of 48 yes/no questions that measure extraversion, neuroticism and psychoticism traits of personality (Eysenck & Eysenck, 1991). Higher scores on the 12 items of a given personality scale indicate a stronger tendency to exhibit that personality trait. Finally, participants reported how many units of alcohol they drank regularly per week.

2.3. Cold pressure test

To induce stress we used the well established cold pressure test (CPT, von Baeyer et al., 2005; Colzato et al., 2008). Participants were asked to immerse a hand in cold water (1–4 °C) for 1.5 min. Pumps circulating the cold water prevented the development of a microenvironment of warmer water around the hand of the participant. Ice cubes were used to cool the water and a perforated plastic separated the ice from the hands of the participants.

2.4. Subjective measures of stress

Subjective indicators of anxiety, nervousness, and feelings of insecurity were assessed once (after the CPT) on a visual analogue scale ranging from 0 to 10.

2.5. General procedure

Prior to the testing session, participants were given the Oragene TM DNA self-collection kit for DNA sampling. After receipt of their DNA, participants were scheduled for testing within a fixed time window (between 1500 and 1800 h) to counter effects of circadian changes in cortisol. Following Colzato et al. (2008), participants were asked to minimize their physical exercise during the hour before the experiment and to refrain from big meals, coffee, tea, drinks with a low pH, chocolate or chocolate milk, psychoactive drugs and alcohol (all variables known to have an influence on cortisol levels) starting from 20:00 the evening before the experiment. Compliance with these instructions was motivated by announcing that saliva samples would be taken. Testing was carried out by lab-assistants, who were naive with respect to the hypotheses and participants' genetic predispositions, in a laboratory in the Institute for Psychological Research of Leiden University.

The study consisted of one session lasting for about 50 min. At every assessment point all physiological stress markers (saliva, HR, SBP and DBP) were measured. The study comprised two parts. In the first part participants were informed that later they would undergo a painful stress procedure. Afterwards, a baseline assessment of physiological measures (−15 min) was followed by the filling out the LSAS, the EPQ-RSS and the rating scale about weekly alcohol consumption.

The second part of the study consisted of the CPT followed by the presentation of a rating scale to obtain subjective measures of stress and two assessments of physiological measures (+15 min and +30 min).

2.6. Sampling and biochemical analysis

Salivary cortisol: Salivary cortisol was sampled three times during the 50-min session at the following time-points: 15 min prior to the CPT and 15 and 30 after the CPT. Saliva samples were obtained using Salivettes (Sarstedt, Rommelsdorf, Germany). Saliva samples were stored at −18 °C before assaying (Dresden LabService GmbH, Dresden, Germany).

Heart rate and blood pressure: Heart rate and systolic and diastolic blood pressure (SBP and DPB) were measured from the non-dominant arm with a OSZ 3 Automatic Digital Electronic Wrist Blood Pressure Monitor (Speidel and Keller).

2.7. DNA laboratory analysis

Genomic DNA was extracted from saliva samples using the Oragene TM DNA self-collection kit following the manufacturer's instructions (DNA Genotek, Inc., 2006). Following Colzato et al. (2010a,b,c), the genotype was scored by two independent readers by comparison to sequence-verified standards. The Val^{66}Met polymorphism (rs 6265) was extracted from whole genome data using PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink). Val^{66}Met was in the equilibrium as stated by Hardy and Weinberg ($p = 0.26$). Moreover, genotype frequencies (Val^{66}Val 65.5%, Val^{66}Met 31.5%, and Met^{66}Met 3%) were similar to those reported in previous studies on Caucasian populations (Gatt et al., 2009; Lang et al., 2005; Elzinga et al., 2010). Individuals who were homozygous for the Met allele were merged with the heterozygous individuals into a group of Met carriers ($n = 39$) and compared to homozygous Val carriers ($n = 59$).

2.8. Statistical analysis

All statistical tests were carried out using SPSS version 17 (Windows). First, statistical analysis of cortisol, heart rate, and blood pressure was subjected to repeated measures gen-

eral linear models (GLMs) to evaluate the effects of time (three sampling time-points), genotype (Val/Val and Met carriers), sex (male and female), and contraceptive-use in women (use and no use). Second, univariate ANOVAs were performed for analyses of personality traits, anxiety, and alcohol consumption differences between genotype groups. Greenhouse-Geisser corrections were applied if sphericity (significant differences in variance between groups) was significant, and only adjusted results are reported. Third, Pearson correlation coefficients were computed between alcohol consumption, cortisol levels (at Time 1), neuroticism, and anxiety scores in order to test whether the rise of cortisol is associated with the amount of weekly alcohol consumption, neuroticism and anxiety levels.

3. Results

3.1. Salivary cortisol

Table 1 provides an overview of the mean score at different time points for cortisol levels. Given that the menstrual cycle and oral contraceptives have been reported to impact salivary cortisol in social stress paradigm (Kirschbaum et al., 1999), we first compared cortisol measures obtained from women who were using oral contraception (OC+, $n = 38$) and from women who did not (OC−, $n = 16$) with those from men. As no reliable differences were obtained, $F(1,52) = 0.94$, $p = 0.31$, $\eta^2 = 0.02$, $F(1,80) = 0.004$, $p = 0.94$, $\eta^2 = 0.001$, $F(1,58) = 0.86$, $p = 0.36$, $\eta^2 = 0.01$, respectively (see Supplementary data), contraceptive-use and gender were not further considered in subsequent analyses (Table 2).

A repeated-measures ANOVA of the total sample, with the cortisol levels at the 3 time points of measurement as dependent variables, revealed a main effect of time, $F(2,184) = 6.12$, $p < 0.01$, $\eta^2 = 0.06$, showing that cortisol levels rose from Times 1 to 3 (see Fig. 1). Interestingly, the main effect of Val^{66}Met polymorphism was not significant ($F < 1$) but interacted significantly with time, $F(2,184) = 3.15$, $p < 0.05$, $\eta^2 = 0.04$. Post hoc t-tests showed that Met carriers had higher cortisol levels at Time 1 compared to Val/Val homozygotes, $t(94) = 2.243$, $p = 0.027$, while the two groups did not differ at Time 2, $t(94) = 0.420$, $p = 0.676$, or Time 3, $t(94) = 0.312$, $p = 0.756$ (see Fig. 1).

3.2. Blood pressure and heart rate

Table 1 provides an overview of the mean scores at different time points for HR, SBP, and DBP. Replicating the findings from our previous study (Colzato et al., 2008), a repeated-measures ANOVA showed that HR decreased from Times 1 to 3 ($F(2,184) = 20.83$, $p < 0.001$, $\eta^2 = 0.18$), while SBP, $F(2,184) = 2.75$, $p > 0.06$, $\eta^2 = 0.02$, and DBP, $F(2,184) = 2.25$, $p > 0.11$, $\eta^2 = 0.02$, did not change throughout the three assessments. Val^{66}Met polymorphism did not reveal a main effect ($F < 1$) and did not interact significantly with time: HR, $F(2,184) = 1.45$, $p > 0.05$, $\eta^2 = 0.03$, SBP and DBP, F's < 1.

3.3. Self-report measures of stress

The analysis of the stress-related self-report items revealed that Met carriers were significantly more anxious,

Table 1 Sample and genotype-specific demographics, self-reported use of alcohol, subjective measures of stress and questionnaires.

Genotype	N	Sex		Age	Alcohol consumption	CPT			LSAS	Personality traits		
		Male	Female		Weekly*	Anxiety***	Nervous***	Insecurity***	Social anxiety*	Extravert	Psychotic	Neurotic
Met carriers	39	19	20	23.1	12.4 ± 10.5	6.6 ± 1.1	6.3 ± 1.2	6.2 ± 1.0	17.5 ± 8.4	9.2 ± 3.0	3.0 ± 2.0	3.5 ± 2.5
Val/Val homozygous	59	27	32	22.3	8.2 ± 8.6	5.5 ± 0.8	5.4 ± 0.8	5.3 ± 0.9	14.0 ± 8.0	9.2 ± 2.5	2.6 ± 2.1	4.0 ± 2.7

Weekly alcohol consumption: weekly number of standard alcoholic drinks.
CPT anxiety: feelings of anxiety during the cold pressor test.
CPT nervous: feelings of nervousness during the cold pressor test.
CPT insecurity: feelings of insecurity during the cold pressor test.
LSAS social anxiety: social anxiety scores as measured by the Liebowitz Social Anxiety Scale.
Personality traits extravert: extraversion scores as measured by Eysenck's personality questionnaire.
Personality traits psychotic: psychoticism scores as measured by Eysenck's personality questionnaire.
Personality traits neurotic: neuroticism scores as measured by Eysenck's personality questionnaire.
* $p < 0.05$.
*** $p < 0.001$.

Table 2 Means ± standard deviations for cortisol, heart rate, systolic (SBP) and diastolic blood pressure (DBP) for the whole sample, Met carriers and Val/Val homozygous individuals.

	Whole sample	Met carriers	Val/Val homozygous
N	98	39	59
Cortisol			
−15 min	8.5 ± 4.8	9.8 ± 5.3*	7.6 ± 4.2*
+15 min	9.1 ± 4.9	9.4 ± 4.1	9.0 ± 5.3
+30 min	10.3 ± 6.6	10.1 ± 5.2	10.5 ± 7.4
Heart rate			
−15 min	75.8 ± 13.6	78.4 ± 13.5	74.1 ± 13.6
+15 min	68.7 ± 11.2	71.4 ± 10.7	67.0 ± 11.3
+30 min	69.5 ± 12.1	70.4 ± 11.6	68.9 ± 12.5
SBP			
−15 min	128 ± 16	129 ± 17	127 ± 15
+15 min	124 ± 16	127 ± 15	122 ± 17
+30 min	124 ± 19	127 ± 22	123 ± 16
DBP			
−15 min	74 ± 11.3	73 ± 10.5	75 ± 11.9
+15 min	74 ± 9.4	74 ± 10.0	74 ± 9.1
+30 min	76 ± 14.2	78 ± 18.1	75 ± 11.0

* Significant group difference $p < 0.05$.

$F(1,97) = 31.74$, $p < 0.0001$, $\eta^2 = 0.25$, more nervous, $F(1,97) = 23.36$, $p < 0.0001$, $\eta^2 = 0.20$, and more insecure, $F(1,97) = 23.32$, $p < 0.0001$, $\eta^2 = 0.19$ s (see Table 1).

3.4. Weekly alcohol consumption

Met carriers consumed significantly more alcohol than Val/Val homozygote, $F(1,97) = 4.44$, $p < 0.05$, $\eta^2 = 0.05$ (see Table 1).

3.5. Social anxiety (LSAS scale)

Met carriers reached higher scores on social anxiety, $F(1,97) = 4.42$, $p < 0.05$, $\eta^2 = 0.04$, than Val/Val homozygotes (see Table 1).

3.6. Eysenck's personality questionnaire (EPQ-RSS)

Met carriers reached comparable scores on neuroticism, extraversion and psychoticism as Val/Val homozygotes, F's < 1 (see Table 1).

3.7. Correlations

In Met carriers, weekly alcohol consumption correlated positively with feelings of anxiety during CPT, $r(39) = 0.283$, $p < 0.05$, while social anxiety score, neuroticism, and cortisol level at Time 1 followed the same trend without reaching significance. In Val/Val homozygotes, no significant correlations were obtained between alcohol consumption, cortisol level at Time 1, neuroticism, and anxiety scores.

4. Discussion

The present study, for the first time, attempted to understand the multifaceted relation between BDNF Val66Met polymorphism, and stress reactivity — as measured in terms of HPA axis reactivity — anxiety, and alcohol consumption. Met carriers reported significantly stronger feelings of anxiety, nervousness, and insecurity during the CPT, and generally higher scores in social anxiety, than Val/Val homozygotes. The same Met carriers consumed significantly more alcohol per week and showed a stronger anticipatory stress response (an anticipatory psychoendocrine response that occurs during the period immediately preceding the onset of exercise or physical stress; Mason et al., 1973). Met carriers did not show higher cortisol levels in response to the CPT, as indicated by the increased levels of salivary cortisol at Time 1, but not at Time 2. These results suggest that cortisol levels increased in response to the information provided before the CPT that they would undergo a painful experience, rather than to the physical-stress procedure itself.

Our results replicate earlier findings by Jiang et al. (2005) and Montag et al. (2010) in showing an association between the Met-allele and higher anxiety in humans. They are also consistent with the observation of Wichers et al.

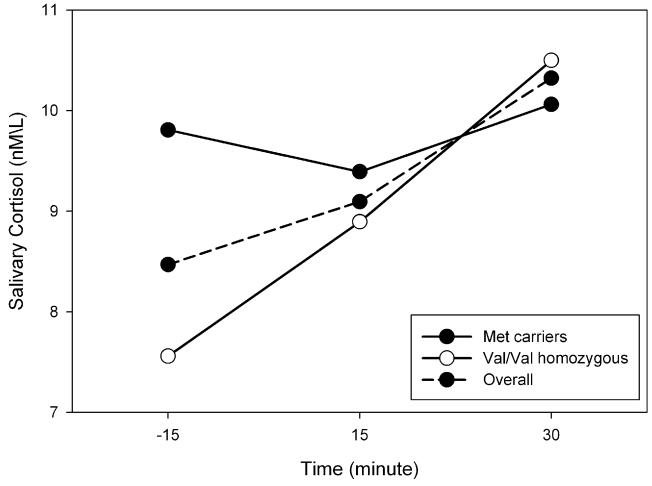

Figure 1 Modulation of salivary cortisol by BDNF Val^{66}Met during the CPT.

(2008) that Met carriers exhibit a more pronounced negative-affect response to social stress than Val/Val homozygotes. Our findings go beyond the study of Shalev et al. (2009) in suggesting that not only social stress (as induced by the Trier Social Stress Test, in which participants role-play in front of two unfriendly interviewers and then carry out a challenging counting task), but also physical stress induces higher cortisol stress responses in association with the BDNF Val^{66}Met polymorphism. Moreover, our study suggests that the BDNF Val^{66}Met polymorphism may not only be associated with alcohol abuse (see Davis, 2008), but may be related to alcohol consumption in the healthy population as well. Finally, we show that, in Met carriers, alcohol consumption correlated positively with feelings of anxiety during the CPT. At this point, we can only speculate that Met carriers may indeed use alcohol to ''control'' the anxiety associated with stressful situation. Given that, in Met carriers, anxiety during CPT, but not neuroticism, correlated with alcohol consumption, we suggest that our findings may be specific to anxiety, rather than to a general neurotic personality trait (at least as measured by the EPQ-RSS questionnaire).

However, we failed to replicate Shalev et al.'s (2009) finding of a gender modulation in the association of BDNF Val^{66}Met polymorphism with HPA axis reactivity to stress: male Val/Val homozygotes showed a greater rise in salivary cortisol than Val/Met heterozygotes, while in female participants there was a trend for the opposite response.

This inconsistency may be explained by the different methodology used by the two studies: the study by Shalev and colleagues used a twice as long protocol as we did, adopted a social rather than physical stressor, and involved eight rather than three samples of salivary cortisol. Even if, gender differences has been found in association with physical stress, both in children and adults (Dixon et al., 2004; Allen et al., 2009), a possible role of the strength of the stress induction — or of the induced stress response — is suggested by the study of Zimmer et al. (2003). They used a version of the CPT, the modified plunge test, which was conducted twice on 2 consecutive trials. The result was a significantly larger cortisol increase in men compared to women, which however was obtained only in the second but not in the first trial. This leaves the possibility that a gender modulation along the lines of Shalev et al. (2009) can be found with a more extreme, or more temporally extended stress induction than we have used in the present study.

A limitation of this study is that, given the correlational nature of the observed relationship between BDNF Val^{66}Met polymorphism, stress reactivity, anxiety, and alcohol consumption, it is not possible to provide a conclusive causal interpretation of the way these factors are related. The interaction between BDNF, anxiety, and drinking behavior seems to be rather dynamic. Indeed, animal and in vitro studies suggest that short-term exposure to alcohol may increase BDNF levels in an effort to maintain homeostasis, but that long-term alcohol use causes both a decrease in BDNF levels in the hippocampus and hippocampal atrophy (for a review, see Davis, 2008). Just like a downward spiral, so one may speculate, anxiety may be reduced by drinking alcohol on the short run but cause more stress on the long run (Sinha, 2008).

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Conflicts of interest

All authors declare that they have no conflicts of interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.psyneuen.2011.04.010.

References

Allen, L.B., Lu, Q., Tsao, J.C.I., Worthman, C.M., Zeltzer, L.K., 2009. Sex differences in the association between cortisol concentrations and laboratory pain responses in healthy children. Gender Med. 6, 193.

Angelucci, F., Brene, S., Mathe, A.A., 2005. BDNF in schizophrenia, depression and corresponding animal models. Mol. Psychiatry 10, 345—352.

Badrick, E., Bobak, M., Britton, A., Kirschbaum, C., Marmott, M., Kumari, M., 2010. The relationship between alcohol consumption and cortisol secretion in an aging cohort. J. Clin. Endocr. Metab. 93, 750—757.

Begliuomini, S., Lenzi, E., Ninni, F., Casarosa, E., Merlini, S., Pluchino, N., et al., 2008. Plasma brain-derived neurotrophic factor daily variations in men: correlation with cortisol circadian rhythm. J. Endocr. 197, 429—435.

Chen, Z.Y., Ling, D.Q., Bath, K.G., Ieraci, A., Khan, T., Siao, C.J., Herrera, D.G., Toth, M., Yang, C.W., Kocsis, J.H., McEwen, B.S., Hempstead, B.L., Lee, F., 2006. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behaviour. Neuropsychopharmacology 31, 110—111.

Colzato, L.S., Kool, W., Hommel, B., 2008. Stress modulation of visuomotor binding. Neuropsychologia 46, 1542—1548.

Colzato, L.S., Pratt, J., Hommel, B., 2010a. Dopaminergic control of attentional flexibility: inhibition of return is associated with the dopamine transporter gene (DAT1). Front. Hum. Neurosci. 14 , doi:10.3389/fnhum.2010.00053.

Colzato, L.S., van den Wildenberg, W., van der Does, W.A.J., Hommel, B., 2010b. Genetic markers of striatal dopamine predict individual differences in dysfunctional, but not functional impulsivity. Neuroscience 170, 782—788.

Colzato, L.S., Waszack, F., Nieuwenhuis, S.T., Posthuma, D., Hommel, B., 2010c. The flexible mind is associated with the Catechol-O-Methyltransferase (COMT) Val158Met polymorphism: evidence for a role of dopamine in the control of task switching. Neuropsychologia 48, 2764—2768.

Corbett, B.A., Schupp, C.W., Levine, S., Mendoza, S., 2009. Comparing cortisol, stress, and sensory sensitivity in children with autism. Autism Res. 2, 39—49.

Dai, X., Thavundayil, J., Gianoulakis, C., 2002. Response of the hypothalamic—pituitary—adrenal axis to stress in the absence and presence of ethanol in subjects at high and low risk of alcoholism. Neuropsychopharmacology 27, 442—452.

Davis, M.I., 2008. Ethanol-BDNF interactions: still more questions than answers. Pharmacol. Ther. 118, 36—57.

Dixon, K.E., Thorn, B.E., Ward, L.C., 2004. An evaluation of sex differences in psychological and physiological responses to experimentally-induced pain: a path analytic description. Pain 112, 188—196.

DNA Genotek Inc., 2006. Oragene TM Product Brochure. DNA Genotek, Inc., Ottawa, ON.

Duman, R.S., Monteggia, L.M., 2006. A neurotrophic model for stress related mood disorders. Biol. Psychiatry 59, 1116—1127.

Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., Weinberger, D.L., 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112, 257—269.

Elzinga, B.M., Ardon, A.M., Heijnis, M.K., de Ruiter, M.B., van Dyck, R., Veltman, D.J., 2007. Neural correlates of enhanced working-memory performance in dissociative disorder: a functional MRI study. Psychol. Med. 37, 235—245.

Elzinga, B.M., Roelofs, K., Tollenaar, M.S., Bakvis, P., van Pelt, J., Spinhoven, P., 2008. Diminished cortisol responses to psychosocial stress associated with lifetime adverse events: a study among healthy young subjects. Psychoneuroendocrinology 33, 227—237.

Elzinga, B.M., Molendijk, M.L., Oude Voshaar, R.C., Bus, B.A.A., Prickaerts, J., Spinhoven, P., Penninx, B.W.J.H., 2011. The impact of childhood abuse and recent stress on serum brain-derived neurotrophic factor and the moderating role of BDNF Val66 Met. Psychopharmacology 214, 319—328.

Erickson, K., Drevets, W., Schulkin, J., 2003. Glucocorticoid regulation of diverse cognitive functions in normal and pathological emotional states. Neurosci. Biobehav. Rev. 27, 233—246.

Eysenck, H.J., Eysenck, S.B.G., 1991. Manual of the Eysenck Personality Scales (EPS Adult). Hodder and Stoughton, London.

Frodl, T., Moller, H.J., Meisenzahl, E., 2008. Neuroimaging genetics: new perspectives in research on major depression? Acta Psychiatry Scand. 118, 363—372.

Gatt, J.M., Nemeroff, C.B., Dobson-Stone, C., Paul, R.H., Bryant, R.A., Schofield, P.R., Williams, L.M., 2009. Interactions between BDNF Val66Met polymorphism and early life stress predict brain and arousal pathways to syndromal depression and anxiety. Mol. Psychiatry 14, 681—695.

Gianoulakis, C., Dai, X., Brown, T., 2003. Effect of chronic alcohol consumption on the activity of the hypothalamic—pituitary—adrenal axis and pituitary β-endorphin as a function of alcohol intake, age and gender. Alcohol. Clin. Exp. Res. 27, 410—423.

Jiang, X., Xu, K., Hoberman, J., Tian, F., Marko, A.J., Waheed, J.F., Harris, C.R., Marini, A.M., Enoch, M.A., Lipsky, R.H., 2005. BDNF variation and mood disorders: a novel functional promoter polymorphism and Val66Met are associated with anxiety but have opposing effects. Neuropsychopharmacology 30, 1353—1361.

Joe, K.-H., Kim, Y.-K., Kim, T.-S., Roh, S.-W., Choi, S.-W., et al., 2007. Decreased plasma brain derived neurotrophic factor levels in patients with alcohol dependence. Alcohol. Clin. Exp. Res. 31, 1—6.

Lang, U.E., Hellweg, R., Kalus, P., Bajbouj, M., Lenzen, K.P., Sander, T., et al., 2005. Association of a functional BDNF polymorphism and anxiety-related personality traits. Psychopharmacology 180, 95—99.

Liebowitz, M.R., 1987. Social Phobia. Mod. Probl. Pharmacopsychiatry 22, 141—173.

Mason, J.W., Hartley, L.H., Kotchen, T.A., Mougey, E.H., Ricketts, N.T., Jones, L.G., 1973. Plasma cortisol and norepinephrine responses in anticipation of muscular exercise. Psychosom. Med. 35, 406—414.

Matsushita, S., Kimura, M., Miyakawa, T., Yoshino, A., Murayama, M., Masaki, T., Higuchi, S., 2004. Association study of brain-derived neurotrophic factor gene polymorphism and alcoholism. Alcohol. Clin. Exp. Res. 28, 1609—1612.

McEwen, B., 1998b. Stress, adaptation, and disease: allostasis and allostatic load. Ann. N. Y. Acad. Sci. 840, 33—44.

Montag, C., Weber, B., Fliessbach, K., Elger, C., Reuter, M., 2009. The BDNF Val66Met polymorphism impacts parahippocampal and amygdala volume in healthy humans: incremental support for a genetic risk factor for depression. Psychol. Med. 39, 1831—1839.

Montag, C., Basten, U., Stelzel, C., Fiebach, C.J., Reuter, M., 2010. The BDNF Val66Met polymorphism and anxiety: support for animal knock-in studies from a genetic association study in human. Psychiatry Res. 179, 86—90.

Murakami, S., Imbe, H., Morikawa, Y., Kubo, C., Senba, E., 2005. Chronic stress, as well as acute stress, reduces BDNF mRNA expression in the rat hippocampus but less robustly. Neurosci. Res. 53, 129—139.

Naert, G., Maurice, T., Tapia-Arancibia, L., Givalois, L., 2007. Neuroactive steroids modulate HPA axis activity and cerebral brain-derived neurotrophic factor (BDNF) protein levels in adult male rats. Psychoneuroendocrinology 32, 1062—1078.

Roceri, M., Cirulli, F., Pessina, C., Peretto, P., Racagni, G., Riva, M.A., 2004. Postnatal repeated maternal deprivation produces age-dependent changes of brain-derived neurotrophic factor expression in selected rat brain regions. Biol. Psychiatry 55, 708—714.

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55—89.

Sertoz, O.O., Tolga Binbay, I., Koylu, E., Noyan, A., Yildirim, E., Elbi Mete, H., 2008. The role of BDNF and HPA axis in the neurobiology of burnout syndrome. Prog. Neuropsychopharmacol. Biol. Psychiatry 32, 1459—1465.

Shalev, I., Lerer, E., Israel, S., Uzefovsky, F., Gritsenko, I., Mankuta, D., Ebstein, R.P., Kaitz, M., 2009. BDNF Val66Met polymorphism is associated with HPA axis reactivity to psychological stress characterized by genotype and gender interactions. Psychoneuroendocrinology 34, 382—388.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry 59, 22—23.

Schüle, C., Zill, P., Baghai, T.C., Eser, D., Zwanzger, P., et al., 2006. Brain-derived neurotrophic factor Val66Met polymorphism and dexamethasone/CRH test results in depressed patients. Psychoneuroendocrinology 31, 1019—1025.

Sinha, R., 2008. Chronic Stress, drug use, and vulnerability to addiction. Ann. N. Y. Acad. Sci. 1141, 105—130.

Song, L., Che, W., Min-Wei, W., Murakami, Y., Matsumoto, K., 2006. Impairment of the spatial learning and memory induced by learned helplessness and chronic mild stress. Pharmacol. Biochem. Behav. 83, 186—193.

Verhagen, M., van der, M.A., van Deurzen, P.A., Janzing, J.G., Arias-Vasquez, A., Buitelaar, J.K., Franke, B., 2010. Meta-analysis of the BDNF val66met polymorphism in major depressive disorder: effects of gender and ethnicity. Mol. Psychiatry 15, 260—271.

Vinberg, M., Trajkovska, V., Bennike, B., Knorr, U., Knudsen, G.M., Kessing, L.V., 2009. The BDNFVal66Met polymorphism: relation to familiar risk of affective disorder, BDNF levels and salivary cortisol. Psychoneuroendocrinology 34, 1380—1389.

von Baeyer, C.L., Piira, T., Chambers, C.T., Trapanotto, M., Zeltzer, L.K., 2005. Guidelines for the cold pressor task as an experimental pain stimulus for use with children. J. Pain 6, 218—227.

Wichers, M., Kenis, G., Jacobs, N., Myin-Germeys, I., Schuers, K., Mengelers, R., et al., 2008. The psychology of psychiatric genetics: evidence that positive emotions in females moderate genetic sensitivity to social stress associated with the BDNF Val66Met polymorphism. J. Abnorm. Psychol. 117, 699—704.

Zimmer, C., Basler, H.D., Vedder, H., Lautenbacher, S., 2003. Sex differences in cortisol response to noxious stress. Clin. J. Pain 19, 233—239.