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SHORT COMMUNICATION

The BDNF Val66Met polymorphism affects HPA-axis reactivity to acute stress

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KEYWORDS

BDNF Val66Met; Hypothalamic pituitary—adrenal axis; Stress reactivity; Saliva cortisol; Heart rate

Summary

Background: Growing evidence suggests that individual differences in HPA-axis reactivity to psychosocial stress are partly due to heritable influences. However, knowledge about the role of specific genetic variants remains very limited to date. Since brain-derived neurotrophic factor (BDNF) not only exhibits neurotrophic actions but is also involved in the regulation of hypothalamic neuropeptides, we investigated the role of a common functional polymorphism within the BDNF gene (BDNF Val66Met) in the context of endocrine and cardiovascular stress reactivity. *Methods:* Healthy male adults (N = 100) were genotyped and exposed to a standardized laboratory stress task (Public Speaking). Saliva cortisol and self-reported mood levels were obtained at 6 time points prior to the stressor and during an extended recovery period. Furthermore, heart rate reactivity as an indicator of sympathetic activation was monitored continuously during the experimental procedure.

Results: We report a small, but significant effect of the BDNF Val66Met polymorphism on stress reactivity. More precisely, carriers of the met-allele showed a significantly attenuated HPA-axis and cardiovascular reactivity to the psychosocial stressor compared to subjects with the val/val genotype. Furthermore, the diminished physiological response in met-allele carriers was also attended by significantly lower self-reported ratings of perceived stress and nervousness. *Conclusion:* Our findings of a diminished endocrine and cardiovascular stress response in healthy male adults is consistent with a previously published study and adds further evidence for a crucial role of the BDNF Val66Met polymorphism in the modulation of stress reactivity. © 2010 Published by Elsevier Ltd.

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Introduction

Activation of the HPA-axis in response to psychosocial stress is characterized by substantial interindividual variations, leading to differences in the ability to maintain homeostasis during challenges. Since alterations of HPA-axis activity have been implicated in the pathogeneses of stress-related disorders (see Plotsky et al., 1998 for review), current research attempts to identify specific factors that contribute to individual differences in the neuroendocrine stress response. Results from twin studies suggesting that reactivity of the HPA-axis elicited by psychosocial stress is partly heritable (e.g. Federenko et al., 2004), stimulated genetic association studies in this field of research.

An attractive candidate gene with potential effects on the neuroendocrine stress response is the brain-derived neurotrophic factor (BDNF) gene, given that BDNF not only exhibits neurotrophic actions but is also highly stress sensitive and involved in the regulation of hypothalamic neuropeptides (see Tapia-Arancibia et al., 2004 for review). In humans, a common functional polymorphism in the BDNF gene (BDNF Val66Met), producing an amino acid substitution (valine to methionine) at codon 66 in the prodomain, impairs intracellular trafficking and activity-dependent secretion of BDNF (Egan et al., 2003). According to the neurotrophic hypothesis of depression, this polymorphism has been extensively studied in the context of stress-related disorders (meta-analyses: Verhagen et al., 2008; Chen et al., 2008), leading to highly inconsistent results. Therefore, numerous genetic association studies in this field of research emphasize the need for endophenotype-strategies (Gottesman and Gould, 2003), for example by exploring genotype-related alterations on a neural and endocrine level. Despite the accumulating evidence for a modulating role of BDNF in HPA-axis activity, to date only one study explicitly investigated the association between the BDNF Val66Met polymorphism and endocrine stress reactivity in a human sample (Shalev et al., 2009). The authors report a gender-dependent effect of the met-allele, pointing to an attenuated cortisol response in male subjects.

Since replication of such initial findings is of extreme importance in the field of genetic associations studies, we investigated the association between BDNF Val66Met genotype and response to a psychosocial stressor in a sample of 100 healthy male adults who participated in a recently published study addressing gene-by-environment interactions on HPA-axis reactivity (Alexander et al., 2009).

Methods

Subjects

One hundred healthy male adults participated in the study (mean age: 23.79 ± 2.7 ; mean body mass index (BMI): 23.18 ± 3.1). Subjects were recruited via announcement in the local newspaper and received \in 40 for participation. Before entering the study, participants completed a structured interview by phone and the German version of the Beck Depression Inventory (BDI, Hautzinger et al., 1994) as well as a detailed questionnaire on mental and physical health status. Current or past mental and/or chronic physical problems as well as consumption of psychotrophic drugs or those exerting influence on HPA-axis functioning were defined as exclusion criteria. To avoid potential confounds due to stratification we included only Caucasian participants with European background who were native German speakers. Subjects gave informed, written consent to participate in the study, which was approved by the Ethics Committee of the German Psychologist Association.

Questionnaires

Prior to testing, the Beck Depression Inventory (BDI, Hautzinger et al., 1994) was administered which contains 21 items measuring severity of depressive symptoms with scores higher than 18 indicating clinically relevant symptoms. Furthermore, all subjects completed the German version of the NEO Fivefactor Inventory (NEO-FFI), a 60-item self-report measure of personality, grouped into five major domains: neuroticism, extraversion, openness to experience, conscientiousness and agreeableness (Borkenau and Ostendorf, 1994).

The psychosocial stress protocol

For stress induction we used the well-established paradigm of Public Speaking which has proven to be a reliable tool to elicit robust cortisol elevations. A detailed description of the experimental procedure has been described elsewhere (Alexander et al., 2009). During the stress paradigm saliva samples were collected to assess changes in cortisol concentrations at 6 time points: baseline (0), after anticipation (+15 min), after speech (+25 min) and 3 times during an extended final relaxation period (+50 min, +75 min, +100 min). During saliva sampling participants rated their emotional state by use of a visual analogue scale ranging from 0 (lowest value) to 16 (highest value). Furthermore, heart rate activity had been monitored continuously within the experimental procedure using a LabLink V System (Coulbourn Instruments, Allentown, PA, USA).

Genotype analysis

DNA was extracted from buccal cells and purification of genomic DNA was performed with a standard commercial extraction kit (MagNA Pure LC DNA Isolation Kit I; Roche Diagnostics, Mannheim, Germany). Genotyping of the BDNF Val66Met polymorphisms was performed by real-time PCR using fluorescence melting-curve detection analysis by means of the Light Cycler System (Roche Diagnostics). A detailed protocol is provided in the supplementary material.

Hormone assays

Saliva samples were obtained using Salivette collection devices (Sarstedt, Rommelsdorf, Germany) and were stored at -20 °C before assaying. Biochemical analysis of free cortisol in saliva was performed using a commercial enzyme-linked immunosorbent assay kit (DRG Instruments GmbH, Marburg, Germany). The analytical sensitivity of the assay is 0.331 nmol/l. All samples were analyzed in duplicates. The intra-assay variation (CV) on three saliva samples of the low (2.76 nmol/l) medium (41.4 nmol/l) and high controls (276 nmol/l) were averaged 0.86, 2.82 and 2.85%,

Table 1

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BDNF Val66Met and endocrine stress reactivity

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Statistical analyses were conducted by comparing met-allele carriers vs. subjects homozygous for the val-allele.				
	BDNF Val66Met genotype			
	val/val (n = 66)	val/met (<i>n</i> = 30)	met/met (<i>n</i> = 4)	р
Age (years)	$\textbf{24.0} \pm \textbf{2.9}$	$\textbf{25.1} \pm \textbf{2.6}$	$\textbf{25.5} \pm \textbf{2.4}$	n.s.
BMI	$\textbf{23.0}\pm\textbf{3.2}$	$\textbf{24.0} \pm \textbf{2.9}$	$\textbf{20.7} \pm \textbf{1.7}$	n.s.
Neuroticism (NEO-FFI)	$\textbf{2.5}\pm\textbf{0.5}$	$\textbf{2.6} \pm \textbf{0.7}$	$\textbf{2.3} \pm \textbf{0.5}$	n.s.
Depression (BDI)	6.1 ± 5.3	$\textbf{5.7} \pm \textbf{5.2}$	$\textbf{4.4} \pm \textbf{2.1}$	n.s.

BMI, body mass index; BDI, Beck Depression Inventory; NEO-FFI, NEO Five-factor Inventory.

respectively. Inter-assay coefficients of variation were 5.44, 7.32, 9.61%, respectively.

Statistical analyses

Analyses were conducted using SPSS 11.5. for Windows (SPSS, Inc., Chicago, IL). All statistical tests were two-tailed with alpha set at p < 0.05. Free saliva cortisol responses, heart rate reactivity and changes in self-reported mood measures were analyzed with a repeated measure analysis of variance (ANOVA) to ensure successful stress induction. To test for effects of BDNF Val66Met genotype on stress reactivity, mixed design analyses of covariance (ANCOVA) were conducted with repeated measures (number of samples), genotype as between subject factor (comparing val/met, met/ met vs. val/val) and baseline cortisol values as covariate. Greenhouse Geisser corrections for repeated measures were calculated when data violated the sphericity assumption. Additionally, area under the response curve (AURC) values were calculated according to the trapezoid rule. In subsequent analyses we conducted additional ANCOVAs to account for effects of potential confounds on cortisol reactivity and included age, BMI, time of awakening, BDI depression and NEO-FFI neuroticism score as covariates in the model. Due to technical problems data on heart rate reactivity was not available from 8 participants, therefore all statistical analyses referring to this variable comprise the remaining 92 subjects. To further ensure stability of results over different statistical approaches, we conducted linear regression analyses to verify significant ANCOVA results.

Results

Sample characteristics

As summarized in Table 1, groups separated by BDNF Val66-Met genotype did not differ significantly with respect to age, BMI, neuroticism and depression scores. The BDNF Val66Met genotype frequencies for BDNF Val66Met did not differ significantly from Hardy–Weinberg expectations ($\chi^2 = 0.06$, df = 1, p = 0.80).

Stress response to Public Speaking

As expected, Public Speaking induced significant elevations of cortisol levels [$F(2.6,256.9) = 21.0, p \le 0.0001, \eta^2 = 0.18$] and heart rate activity [$F(2.4,217.7) = 279.9, p \le 0.0001, \eta^2 = 0.76$]. Kolmogorov-Smirnov test revealed no deviation

from normal distribution concerning cortisol [z = 1.22, p = 0.10] and heart rate reactivity [z = 0.5, p = 0.97] with regard to AURC, therefore all analyses were carried out with raw values. Self-reports during the stress procedure revealed significant elevations of perceived nervousness [F(3.1,305.8) = 76.6, $p \le 0.0001$, $\eta^2 = 0.44$] and stress [F(3.3,331.2) = 51.0, $p \le 0.0001$, $\eta^2 = 0.34$].

Effects of BDNF Val66Met genotype on stress reactivity

Baseline cortisol concentrations did not differ according to the BDNF Val66Met genotype [F(1,98) = 0.4, p = 0.51]. ANCOVA revealed a significant main effect of genotype on cortisol secretion patterns $[F(1,97) = 4.3, p = 0.04, \eta^2 = 0.04]$ Fig. 1a], indicating an attenuated endocrine stress response in met-allele carriers compared to subject homozygous for the val-allele. Similar results were obtained when calculating the main effects of genotype on AURC as dependent variable $[F(1,98) = 4.2, p = 0.04, \eta^2 = 0.04]$. The observed effect remained stable across different statistical approaches as a linear regression analysis confirmed that cortisol AURC can significantly be predicted by BDNF Val66Met genotype $[\beta = -202, t = -2.05, p = 0.04, R^2 = 0.04]$. When analyses were repeated with age, BDI depression score, NEO-FFI neuroticism score and time of awakening as covariates, no changes in the overall results were observed (data not shown).

Findings of an attenuated stress response in met-allele carriers were further confirmed with regard to heart rate reactivity. Baseline heart rate values did not differ as a function of BDNF Val66Met genotype [F(1,90) = 0.9, p = 0.34]. While there was no main effect of genotype on heart rate reactivity [F(1,90) = 0.01, p = 0.37], ANCOVA revealed a significant genotype \times time interaction [F(2.1,188.8) = 3.0, p = 0.01, $\eta^2 = 0.03$; Fig. 1b], again showing that met-allele carriers are characterized by a diminished heart rate activity but only during the speech phase.

Referring to baseline self-reported values, met-allele carriers did not differ from val/val subjects with regard to perceived nervousness [F(1,98) = 0.1, p = 0.74] but reported significantly lower levels of perceived stress [F(1,98) = 5.6, p = 0.02, $\eta^2 = 0.05$]. Concerning reactivity, met-allele carriers reported lower levels of nervousness in response to the stressor compared to val/val subjects (Fig. 1c) as indicated by a significant main effect of BDNF Val66Met genotype [F(1,97) = 6.0, p = 0.02, $\eta^2 = 0.06$] and a significant genotype \times time interaction [F(2.7,264.0) = 4.32, p = 0.001, $\eta^2 = 0.04$]. Furthermore, the same result pattern emerged with regard to self-reported stress levels, where a significant

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(a) 10 -val/val val/met, met/met 8 Mean saliva cortisol (mmol/l +/- SEM) 6 4 2 0 +25 +75 +100 +0 +15 +50 **Collection Times** (b) 120 -val/val val/met, met/met 110 beats per minute) Mean heart rate 100 90 80 70 Speech 0 +15 +25 +50 +75 +100 +0 **Collection Times** 10 (c) val/val val/met, met/met 8 Mean self-reported "nervousness" 6 4 2 0 +15 +25 +50 +75 +100 +0 **Collection Times**

Figure 1 Saliva cortisol (a), heart rate (b) and self-reported nervousness (c) in response to Public Speaking as a function BDNF Val66Met genotype (comparing met-allele carriers vs. val/val subjects).

main effect of genotype [F(1.98) = 5.99, p = 0.03, $\eta^2 = 0.05$, data not shown] points to lower values in met-allele carriers during the experimental procedure.

Discussion

Our results indicate a small, but significant effect of the BDNF Val66Met polymorphism on HPA-axis reactivity to a standar-

dized laboratory stress task in healthy male adults, pointing to an attenuated cortisol response in met-allele carriers when compared to subjects homozygous for the val-allele. Moreover, the same picture emerges with regard to heart rate reactivity as an indicator for sympathetic activation. Therefore, our results provide direct support for a recent study, showing a diminished endocrine and cardiovascular stress response to a slightly different stress paradigm (Trier Social Stress Test) in a group of healthy male subjects (Shalev et al., 2009).

With regard to potential underlying molecular mechanisms of our findings, it is important to note that animal studies consistently report high concentrations of both BDNF and its high affinity receptor trkB mRNA in hypothalamic nuclei and neurons secreting corticotropin releasing hormone (CRH), providing an important fundament for potential autocrine or/and paracrine actions of BDNF at this level (see Tapia-Arancibia et al., 2004 for review). Underpinning the crucial role of BDNF as a stress-responsive messenger, intracerebroventricular BDNF injection in rats induces a gradual increase of CRH in hypothalamic regions and subsequently elevations of plasma adrenocorticotropic hormone (ACTH) and cortisol concentrations 30 min after injection (Givalois et al., 2004). Moreover, acute stress results in significant elevations of BDNF mRNA in hypothalamic regions (Smith et al., 1995; Rage et al., 2002) and close temporal correlations between stress-induced hypothalamic BDNF increase and CRH mRNA variations have been observed (Givalois et al., 2004). Based on these findings, it is tempting to speculate that reduced activity-dependent secretion of BDNF in metallele carriers results in an attenuated BDNF release to stressful situations which in turn leads to a diminished BDNF-induced activation of HPA-axis.

Alternatively, diminished physiological stress reactivity in met-allele carriers might simply reflect stable differences in the perception of stressful situations, since the attenuated endocrine and cardiovascular stress response observed in these subjects was also attended by lower ratings of perceived stress and nervousness. These findings provide support for a recent meta-analysis demonstrating that met-allele carriers exhibit lower values in anxiety-related personality traits like neuroticism (Frustaci et al., 2008) and might therefore respond less intense to stressful environmental challenges.

Beside the strong need for exploring neuronal mechanisms of our findings, future studies should also address combined effects of targeted genetic variants or environmental factors to further extend existing findings regarding the role of BDNF Val66Met polymorphism in stress reactivity. Since a significant interaction between a genetic variation within the serotonin transporter gene (5-HTTLPR) and stressful life events on cortisol responses to acute stress has been demonstrated in the same cohort (Alexander et al., 2009), we also accounted for the role of these factors in post hoc analyses. However, attenuated stress sensitivity in met-allele carriers appeared to be independent of 5-HTTLPR genotype and environmental adversity in our study (data not shown).

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Conflict of interest

None declared.

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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. 2009.12.008.

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