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SHORT COMMUNICATION

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

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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.

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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 €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 Five-factor 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 $-20\,^{\circ}C$ 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%,