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# *BDNF* val$^{66}$met genotype shows distinct associations with the acoustic startle reflex and the cortisol stress response in young adults and children

Diana Armbruster[a,*], Anett Müller-Alcazar[b], Alexander Strobel[a], Klaus-Peter Lesch[c], Clemens Kirschbaum[d], Burkhard Brocke[a]

[a] *Institute of Psychology II, Technische Universität Dresden, Dresden, Germany*
[b] *MSH Medical School Hamburg, University of Applied Science and Medical University, Hamburg, Germany*
[c] *Division of Molecular Psychiatry, Laboratory of Translational Neurobiology, Department of Psychiatry, Psychosomatics, and Psychotherapy, University of Wuerzburg, Wuerzburg, Germany*
[d] *Institute of Psychology I, Technische Universität Dresden, Dresden, Germany*

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ABSTRACT

Brain Derived Neurotrophic Factor (BDNF) is a crucial regulator of neuronal development, organization and function and the val$^{66}$met polymorphism in the *BDNF* gene has been associated with several (endo-) phenotypes of cognitive and affective processing. The *BDNF* met allele is considered a risk factor for anxiety and fear related phenotypes although findings are not entirely consistent. Here, the impact of BDNF val$^{66}$met on two parameters of anxiety and stress was investigated in a series of studies. Acoustic startle responses were assessed in three adult samples ($N_1 = 117$, $N_2 = 104$, $N_3 = 116$) as well as a children sample ($N_4 = 123$). Cortisol increase in response to the Trier Social Stress Test (TSST) was measured in one adult sample ($N_3$) and in the children sample ($N_4$). The *BDNF* met allele was associated with enhanced cortisol responses in young adults ($p = 0.039$) and children ($p = 0.013$). On the contrary, *BDNF* met allele carriers showed a reduced acoustic startle response which reached significance in most samples ($N_1$: $p = 0.004$; $N_2$: $p = 0.045$; $N_3$: n.s., $N_4$: $p = 0.043$) pointing to differential effects of *BDNF* val$^{66}$met on distinct endophenotypes of anxiety and stress-related responses. However, small effect sizes suggest substantial additional genetic as well as environmental contributors.



## 1. Introduction

Brain-derived neurotrophic factor (BDNF) is the most abundant neurotrophin in the brain (Martinowich and Lu, 2008). It is widely expressed and distributed in the CNS and has been found to impact a wide variety of functions, e.g. promoting growth, migration, differentiation and survival of neurons, regulating outgrowth, extension and target-finding of axons and formation of dendrites as well as synaptogenesis (Huang and Reichardt, 2001). A key factor for regulating neuronal survival and cell differentiation during early developmental periods, BDNF is also critical for synaptic plasticity during adulthood (Martinowich et al., 2007).

The *BDNF* gene contains at least eight 5′ exons with respective promoters and transcription start sites as well as one 3′ exon (Adachi et al., 2014). The resulting alternatively spliced RNA transcripts are differentially distributed in various brain regions as well as in diverse cell types and different parts of a neuron (Martinowich et al., 2007). RNA transcripts are first translated into proBDNF, which binds to the intracellular receptor sortilin facilitating correct folding of the mature BDNF domain. BDNF release occurs spontaneously (i.e., constitutive release) or, more frequently, in response to stimuli (i.e., regulated release; Lu and Martinowich, 2008). After being sorted into the regulatory or constitutive pathway, BDNF is trafficked to dendrites or axons, respectively (Lu and Martinowich, 2008). Hence, the binding to sortilin, which is crucial for correct folding of mature BDNF and dendritic trafficking, are under control of the pro-domain (Egan et al., 2003). Thus, genetic variation in the pro-domain such as, for instance, the val$^{66}$met SNP might impact mature BDNF function.

* Corresponding author at: Personality and Individual Differences, Institute of Psychology II, Technische Universität Dresden, 01062 Dresden, Germany. Fax: +49 351 463 36993.
*E-mail address:* diana.armbruster@tu-dresden.de (D. Armbruster).

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In vitro studies have linked the met allele to clear deficits in BDNF trafficking and *regulated* BDNF release (Egan et al., 2003; Martinowich et al., 2007) although *constitutive* release and total BDNF levels appear not to be altered (Chen et al., 2006). However, findings at the behavioral and peripheral physiological level are less clear-cut. In animal studies knock-in mice carrying the met allele displayed increased anxiety-related behaviors (Chen et al., 2006) and pharmacologically increased BDNF signaling was associated with reduced fear responses (Baker-Andresen et al., 2013). Still, others found no difference in anxiety between genetically engineered mice and wild types (e.g., Chourbaji et al., 2004).

In humans, BDNF has been implicated in several neurological and neuropsychiatric disorders (overview: Adachi et al., 2014). A meta-analysis confirmed the association between the met allele and a higher risk for depression, but only in men (Verhagen et al., 2010). The met allele also increases the risk for schizophrenia and eating disorders, but appears to have protective effects with regard to substance-related disorders (meta-analysis: Gratacos et al., 2007). Furthermore, increasing attempts have been undertaken to explore the effects of *BDNF* val$^{66}$met on disorder-related structural and physiological changes. Regarding anxiety, met allele carriers displayed increased amygdala activity and decreased activation in the subgenual anterior cingulate cortex during fear conditioning as well as higher activity in brain regions belonging to the fear network during extinction (Lonsdorf et al., 2014). The met allele was also associated with a more pronounced generalization of conditioned fear (Mühlberger et al., 2014) and in women with a stronger activation of the right amygdala in response to emotional pictures (Montag et al., 2008). Also, smaller volumes of the right amygdala and the para-hippocampal regions have been found in healthy met allele carriers (Montag et al., 2009).

Anxiety disorders and depression are closely interlinked with changed stress reactivity. In turn, while BDNF co-regulates hypothalamus pituitary adrenal (HPA) axis activity, BDNF expression is also impacted by stress exposure resulting in a complex interactional relationship. Bennett and Lagopoulos (2014) point out that in interaction with glucocorticoids and mineralocorticoids, BDNF mRNA and protein is decreased in some brain regions (e.g., parts of the hippocampus) but increased in others (e.g., the basolateral amygdala) in response to stress. The resulting changes in dendritic spine density run parallel to these alterations suggesting differences in *BDNF* gene transcription as its major cause although glucocorticoids and mineralocorticoids might also have further downstream effects.

*BDNF* val$^{66}$met has been associated with differences in stress responses in healthy individuals, albeit not with entirely consistent results. Shalev et al. (2009), employing the Trier Social Stress Test (TSST), reported sex-dependent results: male val/val homozygotes showed a larger cortisol response than met allele carriers while in women a trend for the opposite pattern emerged. Similarly, in an all-male sample, val/val homozygotes had a larger cortisol response to a public speaking paradigm (Alexander et al., 2010). However, Tsuru et al. (2014) reported enhanced cortisol reactions in met/met homozygotes of both sexes in response to the TSST while there was no effect of *BDNF* val$^{66}$met on cortisol responses after a physical stressor (i.e., electrical stimulation). Still, the anticipation of a physical stressor (i.e., cold pressure test) resulted in enhanced cortisol levels in met allele carriers although, again, there were no differences after the actual physical stress (Colzato et al., 2011).

The relationship of BDNF and HPA axis activity has also been investigated in clinical samples. In depressed patients, met/met homozygotes showed enhanced cortisol and adrenocorticotropic hormone (ACTH) responses after the dexamethasone/CRH test (Schüle et al., 2006). *BDNF* val$^{66}$met also modulated the impact of severe real life stressors (i.e., child abuse) on serum BDNF levels. In adults with lifetime major depression, met allele carriers with a history of childhood abuse had reduced serum BDNF levels, while met carriers who had not been exposed to childhood abuse had higher levels in comparison to val/val genotypes (Elzinga et al., 2011). Also, met allele carriers with a high familiar risk for affective disorders had higher whole blood BDNF and higher evening cortisol levels indicating a risk for enhanced stress responses (Vinberg et al., 2009).

In sum, while in vitro studies produced clear evidence that the met allele results in deficient BDNF trafficking and release, behavioral and physiological findings are equivocal. Here, we investigated in multiple samples whether replicable patterns of *BDNF* val$^{66}$met influence can be determined in two indicators of negative emotionality: the acoustic startle reflex and the cortisol stress response. Both paradigms are well-established and used for determining physiological features of emotional reactions and the induction of psycho-social stress, respectively. The acoustic startle reflex (ASR) in response to an unexpected intense stimulus consists of a number of very fast contractions of skeletal and facial muscles, closing of the eyes, acceleration of the heart rate and an arrest of ongoing behaviors (Koch, 1999). ASR magnitudes vary widely between subjects (Blumenthal et al., 2004) and show considerable heritability (Anokhin et al., 2007). They are also positively related to anxiety scores (e.g., Poli and Angrilli, 2015). Startle magnitudes can be further modulated by additional positive stimuli which result in a decreased startle reflex (pleasure-attenuated startle, PAS) while negative stimuli, particularly highly arousing ones, lead to a stronger response (fear-potentiated startle; FPS; Koch, 1999).

Cortisol increase after stress is the result of a neuro-hormonal cascade consisting of CRH release from the paraventricular nucleus (PVN) of the hypothalamus followed by stimulation and release of ACTH from the anterior pituitary. ACTH subsequently stimulates the release of glucocorticoids from the adrenal glands. Cortisol as the major human stress hormone influences numerous physiological systems (Kino and Chrousos, 2005) and serves as a negative feedback signal to the PVN and the pituitary (Kunugi et al., 2010). As with the ASR, inter-individual variation of the cortisol stress response is extensive with genetic and environmental factors as contributors (Kirschbaum and Hellhammer, 1999).

Based on previous albeit not entirely consistent findings of increased anxiety-related behavior in met allele carriers, we expected larger startle magnitudes and more pronounced cortisol stress responses in these genotypes. Three samples of young adults and one children sample were examined providing the additional opportunity to investigate the effect of *BDNF* depending on developmental stage.

## 2. Materials and methods

### 2.1. Participants

Three samples of young adults and one children sample of German/Middle European ancestry were recruited during the course of a series of research projects. Prior to arranging lab appointments, a telephone screening was conducted in order to assess physical and mental health. In case of the children sample the telephone interview was conducted with one of the parents. Inclusion criteria were normal or corrected to normal vision, no hearing deficits of any kind, absence of reported current psychological problems and past diagnoses of neuro-psychiatric disorders as well as absence of severe physical impairment or illness (e.g., cardio-vascular diseases, diseases of the respiratory tract, liver diseases, diseases of the kidneys and the urinary passage, intestinal diseases and disorders, metabolic disorders, muscle diseases, blood diseases, severe infectious diseases, severe allergies, autoimmune diseases, thyroid diseases, head injuries). No participant used drugs and no