International Journal of Neuropsychopharmacology (2012), **15**, 1229–1239. © CINP 2011
doi:10.1017/S1461145711001763

ARTICLE

CINP

Children under stress – *COMT* genotype and stressful life events predict cortisol increase in an acute social stress paradigm

Diana Armbruster[1], Anett Mueller[2], Alexander Strobel[1], Klaus-Peter Lesch[3], Burkhard Brocke[1] and Clemens Kirschbaum[4]

[1] *Institute of Psychology II, Technische Universitaet Dresden, Dresden, Germany*
[2] *Department of Psychology, Stony Brook University, Stony Brook, NY, USA*
[3] *Molecular Psychiatry, Laboratory of Translational Neurobiology, Department of Psychiatry, Psychosomatics and Psychotherapy, University of Wuerzburg, Wuerzburg, Germany*
[4] *Institute of Psychology I, Technische Universitaet Dresden, Dresden, Germany*

Abstract

Dopamine and norepinephrine are key regulators of cognitive and affective processes. The enzyme catechol-*O*-methyltransferase (COMT) catabolizes catecholamines and the *COMT* Val[158]Met polymorphism has been linked to several neuropsychiatric variables. Additionally, stressful life events (SLEs) contribute substantially to affective processes. We used the stress-induced activation of the hypothalamic-pituitary-adrenal axis to investigate the effects of *COMT* and SLEs on the cortisol response in 119 healthy children (8–12 yr). Saliva cortisol was measured during and after the Trier Social Stress Test for Children. SLEs were assessed with a standardized interview with one of the children's parents. Linear regression analysis revealed a significant effect for *COMT*, with Met allele carriers showing a higher cortisol response ($\beta = 0.300$, $p = 0.001$). In turn, more SLEs lead to a less pronounced cortisol increase ($\beta = -0.192$, $p = 0.029$) probably indicating increased resilience. Our results further underscore the essential and differential role of genetic variation and environmental factors on stress responsivity.

Received 8 July 2011; Reviewed 8 August 2011; Revised 3 November 2011; Accepted 10 November 2011; First published online 12 December 2011

Key words: Children, *COMT* Val[158]Met, cortisol stress response, HPA axis, stressful life events.

Introduction

Stress is an inevitable part of everyday life. Thus, an organism must respond with an adaptive set of reactions in order to cope with a potentially threatening situation and – in the long run – to restore homeostasis. However, there are considerable inter-individual differences in the response to challenges, which are probably based on complex gene × gene and gene × environment interactions (McClearn, 2006). In mammals, activation of the hypothalamic-pituitary-adrenal (HPA) axis constitutes one of the main pathways of the stress response, resulting in the release of corticotropin-releasing hormone (CRH) from the paraventricular nucleus (PVN) of the hypothalamus with a subsequent stimulation of adrenocorticotropic hormone (ACTH) and the secretion of glucocorticoids. Cortisol as the major human stress hormone influences numerous physiological systems, including central nervous system function, metabolism, cardiovascular function, immune system, muscle tissue and bones (Kino & Chrousos, 2005). HPA axis reactivity varies widely even in healthy individuals with genetic as well as environmental factors as likely contributors (Kirschbaum *et al.* 1992; Linkowski *et al.* 1993). In this context, the modulation of the HPA axis by additional neural structures, including the brainstem, the amygdala, the hippocampus and the medial prefrontal cortex (PFC; e.g. Dedovic *et al.* 2009*a*; Jankord & Herman, 2008) and a number of neurotransmitters innervating these brain regions, suggests several promising candidate genes.

Dopamine and norepinephrine are among those regulators of the stress response (Feldman &

Address for correspondence: Dr D. Armbruster, Personality and Individual Differences, Institute of Psychology II, Technische Universitaet Dresden, 01062 Dresden, Germany.
Tel.: +49 351 463 36997 *Fax*: +49 351 463 36993
Email: armbruster@psychologie.tu-dresden.de

Weidenfeld, 2004; Sullivan & Dufresne, 2006). In turn, stress has also been found to impact dopaminergic function (Arnsten & Goldman-Rakic, 1998; Murphy *et al.* 1996). Variations in the gene coding for catechol-*O*-methyltransferase (COMT), the enzyme facilitating metabolic degradation of catecholamines (Chen *et al.* 2004; Weinshilboum *et al.* 1999), have been suggested to impact stress reactivity (Jabbi *et al.* 2007; Zubieta *et al.* 2003). COMT exists in two isoforms: soluble, predominantly expressed in tissues such as liver, blood and kidney; membrane-bound, mainly expressed in the brain, particularly in the PFC (Chen *et al.* 2004; Karoum *et al.* 1994; Matsumoto *et al.* 2003; Tenhunen *et al.* 1993). The *COMT* gene is located on chromosome 22q11 and contains several single nucleotide polymorphisms (SNPs) including a G/A substitution (rs4680) at codon 158, which results in the substitution of valine (Val) and methionine (Met). The Val^{158}Met SNP changes the thermal stability and activity of COMT. Met/Met homozygotes exhibit 35–50% less COMT efficiency in the PFC at 37 °C compared to Val/Val homozygotes (Chen *et al.* 2004). Thus, the Met allele probably leads to enhanced catecholaminergic activity (Karoum *et al.* 1994; Tunbridge *et al.* 2004) and might therefore impact cortical function.

The *COMT* Val allele has been linked to insufficient prefrontal activation during tasks measuring cognitive control and working memory (Blasi *et al.* 2005; Dumontheil *et al.* 2011; Egan *et al.* 2001; Winterer *et al.* 2006) and to poorer performance in prefrontally mediated tasks (Barnett *et al.* 2007; Goldberg *et al.* 2003; Joober *et al.* 2002; Malhotra *et al.* 2002). Conversely, the *COMT* Met allele has been associated with heightened scores of negative emotionality measured with self-reports (Lang *et al.* 2007; Reuter & Hennig, 2005; Stein *et al.* 2005), although inconsistent findings exist (Wray *et al.* 2008). In addition, Met allele carriers show an increased proneness for the development of anxiety disorders (Enoch *et al.* 2003; Olsson *et al.* 2005; Woo *et al.* 2004) and depression (Aberg *et al.* 2011). Psychophysiological studies support these findings: the Met allele resulted in elevated startle responses (Armbruster *et al.* 2011; Montag *et al.* 2008) and in increased limbic and prefrontal activation in response to negative stimuli in functional magnetic resonance imaging (fMRI) studies (Drabant *et al.* 2006; Rasch *et al.* 2010; Smolka *et al.* 2005; Williams *et al.* 2010). A recent meta-analysis on fMRI data concluded that Val allele carriers show advantages in emotional but disadvantages in cognitive paradigms while the opposite pattern applies to Met allele carriers (Mier *et al.* 2010). This trade-off had been earlier termed the warrior/worrier model (Goldman *et al.* 2005; Stein *et al.* 2006).

However, while there are numerous studies on emotional regulation, fewer studies have investigated the link between *COMT* and the stress response in general and HPA axis reactivity in particular. Recently, Jabbi *et al.* (2007) reported higher plasma epinephrine levels and elevated subjective stress ratings in response to a modified version of the Trier Social Stress Test (TSST) in *COMT* Met/Met homozygotes. Furthermore, an interaction effect between *COMT* Val^{158}Met and a functional variable number of tandem repeats (VNTR) polymorphism in the dopamine transporter gene (*DAT1* VNTR) – but no *COMT* main effect – was found in response to a public speaking paradigm in healthy young men (Alexander *et al.* 2011). Met/Met homozygotes, who also carried the *DAT1* 10R/10R genotype, showed a stronger cortisol stress response and impaired recovery. Met/Met homozygotes also showed a more pronounced secretion of ACTH and cortisol than Val allele carriers after administration of the opioid antagonist naloxone (Oswald *et al.* 2004). Opioid blockade was chosen because *COMT* Val^{158}Met had been previously linked to changes in μ-opioid receptor binding potential, which, in turn, lead to differences in pain responses (Zubieta *et al.* 2003). *COMT* Val^{158}Met has also been associated with post-traumatic stress disorder (PTSD) after the experience of severe stressors. In survivors of the Rwandan genocide, *COMT* Val allele carriers showed a typical dose–response relationship while Met/Met homozygotes exhibited a heightened PTSD risk independently of the severity of traumatic load (Kolassa *et al.* 2010).

Thus, although overall the *COMT* Met allele seems to be associated with deficits in the stress response, results are not entirely consistent. In addition, the existing studies mainly focus on adult populations. Since genetic variations exist from the very beginning of development it seems important to determine whether such differences are already present in younger individuals. Recently, Walder *et al.* (2010) reported effects of *COMT* on changes in morning cortisol levels in healthy and at-risk adolescents. As expected, there was an increase in cortisol secretion over the course of 1 yr, which was further modulated by *COMT*. Met/Met homozygotes showed a marked increase while there was no increase in Val/Val individuals. In sum: (*a*) there are numerous studies on the effects of *COMT* on negative emotionality but only few studies on the stress response, non of which was conducted in children; (*b*) COMT is expressed in the PFC, a region that has been found to be involved in HPA axis regulation; (*c*) COMT catabolizes dopamine and norepinephrine, which have been reported to regulate HPA axis activity.

As there is evidence from animal studies that the brain-derived neurotrophic factor (BDNF) influences structure and function of the dopaminergic system (Horger *et al.* 1999; Hyman *et al.* 1991; Narita *et al.* 2003; Spina *et al.* 1992), the *BDNF* Val[66]Met SNP was also included, mainly to investigate possible *COMT* × *BDNF* interactions. Furthermore, there is already a number of studies showing that variation in this gene is associated with HPA axis reactivity (e.g. Colzato *et al.* 2011; Elzinga *et al.* 2011; Schule *et al.* 2006; Shalev *et al.* 2009; Vinberg *et al.* 2009). Moreover, animal studies provided evidence that early life stress in turn decreases BDNF expression, resulting in neuronal atrophy and degeneration in the cortex and the hippocampus (Murakami *et al.* 2005; Roceri *et al.* 2002; Song *et al.* 2006).

In addition to genetic variation, stressful life events (SLEs) also contribute to the long-term regulation of the stress response (Tarullo & Gunnar, 2006). Furthermore, exposure to SLEs results in an enhanced risk of developing neuropsychiatric disorders (Paykel, 2003). Particularly, SLEs during critical stages of early development might lead to persisting harmful effects over the course of life. For instance, early social stressors (i.e. low maternal care) in rats lead to increased stress responses in adult individuals via epigenetic processes (Weaver, 2007). Consistently, the crucial role of early SLEs has also been confirmed in humans (Fumagalli *et al.* 2007; Kaufman *et al.* 2000). Additionally, gene × environment interactions have been reported, although not always consistently. With regard to *COMT*, there are, for instance, reports of interaction effects with: (*a*) cannabis use on the later development of psychosis with Val allele carriers at greater risk (Caspi *et al.* 2005); (*b*) maternal cigarette smoking during pregnancy on aggressive behaviour at ages 15 and 20 yr, with Val/Val homozygotes displaying the greatest risk (Brennan *et al.* 2011); (*c*) childhood abuse and anger traits in adulthood, again with Val allele carriers at greater risk (Perroud *et al.* 2010) or (*d*) less sufficient parenting practices, resulting in more alcohol consumption during adolescence in Met/Met homozygotes (Laucht *et al.* 2011).

In order to further examine genetic and environmental underpinnings of stress reactivity, we investigated a sample of children to discover whether: (1) *COMT* Met allele carriers already show a heightened cortisol stress response during childhood; (2) past SLEs affect the cortisol response in this age group; (3) there are *COMT* × SLE interaction effects; (4) *BDNF* Val[66]Met affects the stress response independently or jointly with *COMT* and SLEs.

Method

Participants

All participants were of German/Middle European ancestry and originally consisted of 52 girls and 71 boys. Of these, 122 participants were successfully genotyped for *COMT* and *BDNF*. From the remaining sample, two participants had to be excluded due to extreme cortisol responses (see below) and for one participant cortisol data were not available for the crucial measurement points to calculate cortisol increase, leaving 52 girls and 67 boys for the final sample (mean age 9.32 yr, S.D. = 1.03, range 8–12 yr). All participants were reported to be in good health. The children were screened for psychiatric or neurological disorders or treatment in a telephone interview with their parents before participation. In addition, a semi-structured interview was conducted with one of the parents in order to assess children's SLEs (for details, see below). As part of this interview medical problems were determined and participants whose parents reported a history of mental illness would have been excluded. Participants and their parents were informed about the aims of the study and parents gave written informed consent. The study design was approved by the Ethics Committee of the German Psychological Association.

TSST psychosocial stress protocol

An age appropriate version of the TSST (Kirschbaum *et al.* 1993) for the induction of psychosocial stress was adapted which has been described in details elsewhere and which has been found to elicit a strong and reliable cortisol response [Trier Social Stress Test for Children (TSST-C); Buske-Kirschbaum *et al.* 1997]. This standardized laboratory stressor consists of a preparation period (3 min), followed by a speech task (5 min), during which children had to finish telling a story, and a mental arithmetic task (5 min) to be performed in front of an audience.

Cortisol analysis

Salivary cortisol samples were obtained using 'Salivettes' (Sarstedt; Germany) and kept at −20 °C until analysis. Samples were repeatedly collected for determination of the biologically active 'free' fraction of cortisol before onset of the stress sessions, as well as 2, 10, 20 and 30 min after cessation of stress. Salivary cortisol samples were prepared for biochemical analysis by centrifuging at 1800 *g* for 5 min, which resulted in a clear supernatant of low viscosity. Salivary free cortisol concentrations were determined

by employing a chemiluminescence immunoassay with high sensitivity of 0.16 ng/ml (IBL, Germany). Intra- and inter-assay coefficients of variation were $<8\%$.

Life history calendar

The life history calendar (LHC) is a semi-structured interview method for collecting detailed retrospective data about life events and activities (Axinn *et al.* 1999; Freedman *et al.* 1988).

Validity and reliability of the LHC is enhanced through its use of memory cues. It elicits easily recalled memories and uses this information to aid the retrieval of less easily recalled information. The LHC uses a calendar format, which combines chronological and theme-based structures that support sequencing as well as parallel retrieval approaches. Agreement between retrospectively obtained LHC data and data obtained 5 yr earlier about the respondent's then current situation have been found to range from 72 to 92% (Freedman *et al.* 1988) and, over a 3-yr period, Caspi *et al.* (1996) found 90% agreement. Based on the interview data, a cumulative score representing the total number of reported SLEs can be calculated. However, since the impact of any event might vary between different individuals, parents were also asked to rate each SLE according to the following classification, mild = 1, moderate = 2 or severe = 3, allowing the calculation of a weighted SLE score (SLE-W). Additionally, the LHC data can be grouped in the following subcategories: (1) illness; (2) family problems (e.g. separation or divorce of parents, severe conflicts with parents or siblings, new step-parent); (3) problems related to schooling; (4) negative socioeconomic circumstances; (5) social problems with peers (e.g. end of important friendship); (6) disaster (e.g. severe accidents, fires, floods); (7) death of significant others; (8) other.

Procedure

After a telephone interview with the parents of participating children regarding basic inclusion criteria (e.g. age, health or medication), participants were scheduled for a laboratory session starting between 14:00 and 16:00 hours. Upon arrival, participants were given an overview about the study goals and protocol. The children were accompanied by a parent who stayed with the child during most resting phases and the preparations for the experiments. All participants were instructed that they could decline to participate at any time. After a short resting period, children first underwent a startle experiment (data not reported). The LHC was then conducted with the parent by a trained interviewer (D.A.) while the child had a further resting period of 30 min, after which the TSST-C was conducted. Thus, all TSST-Cs were performed between 15:45 and 18:00 hours. To assess cortisol levels, saliva samples were obtained before and after the startle experiment as well as 2 min before and 2, 10, 20 and 30 min after the TSST-C. A final saliva sample was obtained for later DNA extraction. Participants were subsequently debriefed, reimbursed for participation and thanked.

Genotyping

For genotyping, DNA was isolated from saliva samples using the ORAgene DNA Extraction kits (DNA Genotek, Canada). *COMT* and *BDNF* genotypes were determined by polymerase chain reaction (PCR) followed by digestion of the PCR products and agarose gel size fractionation as described in detail elsewhere (COMT: Armbruster *et al.* 2011; BDNF: Hünnerkopf *et al.* 2007).

Statistical analysis

All analyses were performed using SPSS for Windows 15.0 (SPSS Inc., USA).

Cortisol variables were examined for outliers using boxplots. Two participants were excluded: one with an extremely strong cortisol increase after the TSST-C of 51.03 nmol/l and the other with a very atypical cortisol time course manifesting itself in a marked cortisol decrease of -12.14 nmol/l. In order to determine whether the TSST-C did result in a sufficient cortisol increase, a repeated-measures analysis of variance (ANOVA) was performed with the six measure points as within subject variable. *COMT* and *BDNF* genotype were entered as independent between-subject variables for a first assessment of genotype group differences. A difference score was then computed between cortisol concentrations 20 min after cessation of stress (when cortisol levels had reached their peak) and immediately before the TSST-C (raw variables). A linear regression analysis was performed with *COMT*, *BDNF* and SLE-Ws as predictors and the cortisol difference score as the dependent variable. In order to assess gene × gene and gene × environment interactions, a moderated regression was conducted. Beforehand, SLE-W as a continuous predictor was centred and *COMT* as threefold categorial predictor was recoded as two variables according to the recommendations of West *et al.* (1996). In addition, the products of the transformed independent variables were calculated and entered (West *et al.* 1996). Since

older children might have experienced more SLEs and thus age might confound this predictor, correlations between age and the number of reported SLEs ($p = 0.991$) and the weighted SLEs ($p = 0.552$) were assessed. Since there were no significant correlations, age was excluded as a possible predictor. Furthermore, an additional regression analysis was performed with *COMT*, *BDNF* and the SLE subcategories as predictors to investigate possible differential effects of the various types of SLEs (e.g. illness, death of significant others, family problems).

Results

Genotype frequencies

The genotype frequencies of the *COMT* Val^{158}Met were 27.7% ($n = 33$) for Val/Val, 49.6% ($n = 59$) for Val/Met and 22.7% ($n = 27$) for Met/Met. The genotype frequencies of the *BDNF* Val^{66}Met were 62.2% ($n = 74$) for Val/Val, 35.3% ($n = 42$) for Val/Met and 2.5% ($n = 3$) for Met/Met. *BDNF* Met/Met and Val/Met individuals were grouped together for statistical analysis. Age and sex did not differ by *COMT* (age: ANOVA, $p = 0.323$; sex: χ^2 test, $p = 0.521$) or *BDNF* (age: ANOVA, $p = 0.765$; sex: χ^2 test, $p = 0.254$). The genotypes were in Hardy–Weinberg equilibrium (*COMT*: $p = 0.949$; *BDNF*: $p = 0.295$).

Impact of COMT on the cortisol response

ANOVA revealed a significant time effect indicating that the TSST-C had led to significant changes in cortisol levels ($F_{1.66,184.56} = 47.62$, $p < 0.001$, $\eta^2 = 0.300$). In addition, there was a main effect of *COMT* genotype ($F_{2,114} = 3.79$, $p = 0.026$, $\eta^2 = 0.064$) with Val/Val homozygotes showing the smallest and Met/Met homozygotes the strongest cortisol response while heterozygotes fell in between (Fig. 1). Contrast analyses revealed a significant difference between Val/Val and Met/Met ($p = 0.007$), while the differences between Val/Met and Met/Met ($p = 0.120$) and Val/Met and Val/Val ($p = 0.122$) fell short of reaching significance.

Linear regression ($R = 0.350$) also revealed a significant effect for *COMT* genotype ($p = 0.001$) with the Met allele being associated with a stronger cortisol response ($\beta = 0.300$). The average cortisol increase was more than twice as high in Met/Met compared to Val/Val homozygotes (8.379 nmol/l *vs.* 2.907 nmol/l; Figs. 1 and 2) while Val/Met heterozygotes showed intermediate levels (4.351 nmol/l; Figs. 1 and 2). This effect was not due to baseline differences before the TSST-C (ANOVA, $p = 0.658$).

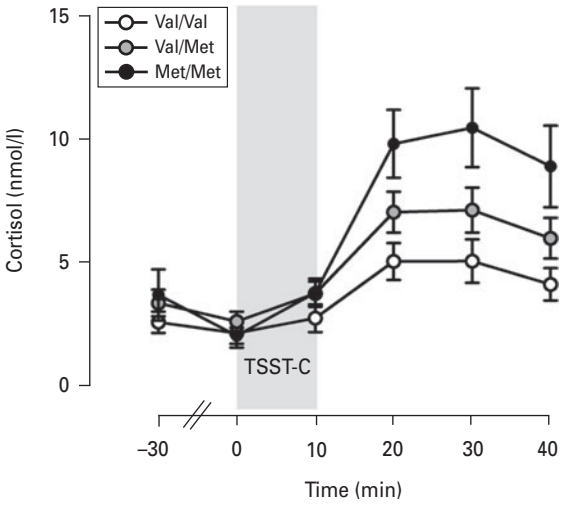

Fig. 1. Time course and effect of catechol-*O*-methyltransferase (mean ± S.E.M.) on salivary cortisol levels in response to the Trier Social Stress Test for Children (TSST-C).

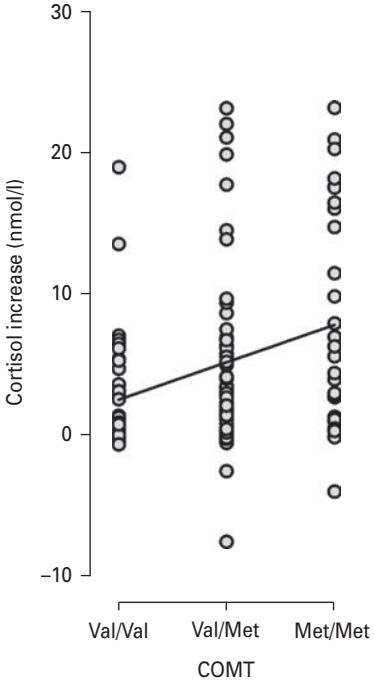

Fig. 2. Effect of catechol-*O*-methyltransferase (*COMT*) on cortisol increase. Scatterplots of cortisol increase as a function of *COMT* genotype.

Impact of SLEs on the cortisol response

The average number of SLEs as reported by the parents was 7.28 (S.D. = 4.10, range 0–21) and the average score of SLE-Ws was 12.23 (S.D. = 6.71, range 0–35). There was no difference between the sexes and

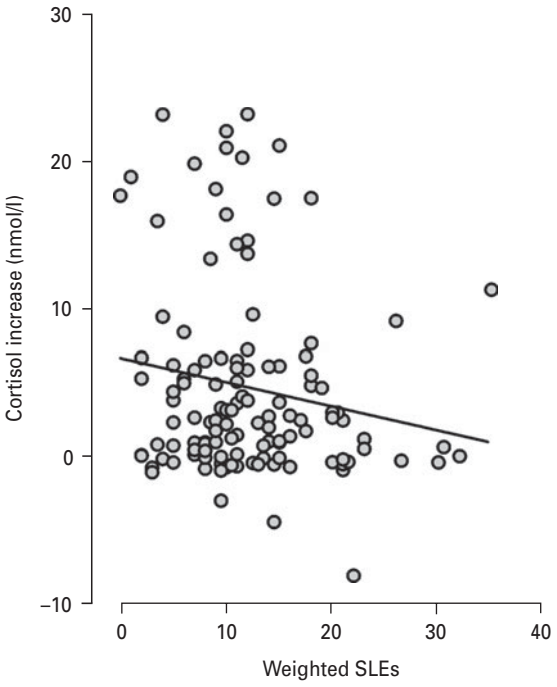

Fig. 3. Effect of stressful life events (SLEs) on cortisol increase. Scatterplots of cortisol increase as a function of weighted SLEs.

the *COMT* genotype groups regarding SLE-Ws (sex: ANOVA, $p=0.233$; *COMT*: ANOVA, $p=0.727$) and, as reported in detail above, SLEs were also unrelated to age ($p \geqslant 0.552$). There was a significant effect for SLE-Ws: the more SLE-Ws had been reported the smaller was the cortisol increase ($p=0.029$; $\beta=-0.192$; Fig. 3). Again, this effect was not due to baseline differences since there was no influence of SLE-Ws on cortisol levels before the TSST-C (linear regression, $p=0.337$).

Regarding the differential effects of various types of life events on the cortisol stress response, the most pronounced effect was found for death of significant others, which, however, failed to reach the conventional significance level by a narrow margin ($p=0.054$, $\beta=-0.180$). All other SLE subcategories did not predict cortisol stress response (all $p \geqslant 0.127$). Furthermore, moderated multiple regression revealed no SLE × *COMT* genotype interaction effect (all $p \geqslant 0.494$).

Impact of BDNF on the cortisol response

Repeated-measurements ANOVA revealed no significant effect of *BDNF* ($p=0.209$) on cortisol levels over time. However, in regression analyses, there was a significant *BDNF* effect on cortisol increase ($p=0.005$, $\beta=0.245$) with Met allele carriers showing a stronger cortisol response. However, moderated regression revealed no SLE × *BDNF* interaction ($p=0.133$) or *COMT* × *BDNF* interaction ($p=0.587$). The three-way interaction *COMT* × *BDNF* × SLE was also not significant ($p=0.136$).

Discussion

We found that *COMT* Val[158]Met genotype and (weighted) SLEs impacted the cortisol stress response in children, but no interaction was found between the two factors. The presence of the *COMT* Met allele led to a stronger cortisol response, while the occurrence of more SLEs resulted in a less pronounced cortisol increase after the TSST-C. These effects were not due to differences in cortisol baseline levels. The main effect of *COMT* is in line with findings indicating that the Met allele is (*a*) a risk factor for deficits in emotional regulation and mood disorders and (*b*) associated with heightened HPA axis activity. The *COMT* Met allele has been linked to insufficient affective regulation, especially anxiety-related traits and disorders (Enoch *et al.* 2003; Mier *et al.* 2010; Olsson *et al.* 2005; Rasch *et al.* 2010; Stein *et al.* 2005; Williams *et al.* 2010; Woo *et al.* 2004) while the Val allele has been associated with less efficient PFC activation during cognitive control and working memory and poorer performance on prefrontally mediated tasks (Dumontheil *et al.* 2011; Egan *et al.* 2001; Mier *et al.* 2010).

This trade-off between enhanced anxiety proneness but better cognitive performance in Met allele carriers and increased resilience but poorer performance in executive tasks in Val allele carriers has been summarized as the warrior/worrier model (Goldman *et al.* 2005; Stein *et al.* 2006).

So far, fewer studies have investigated the link between *COMT* and stress reactivity. Existing findings also mainly suggest the Met allele as a risk factor for deficits in stress responses. Met/Met homozygotes showed higher plasma epinephrine levels and higher subjective stress ratings after confrontation with a psychosocial stressor (Jabbi *et al.* 2007). Furthermore, an interaction effect has been reported between *COMT* Val[158]Met and *DAT1* VNTR. *COMT* Met/Met homozygotes also carrying the *DAT1* 10R/10R genotype showed a stronger cortisol stress response and impaired recovery (Alexander *et al.* 2011). Met/Met homozygotes also responded with greater ACTH and cortisol secretion after the administration of an opioid antagonist (Oswald *et al.* 2004). Additionally, *COMT* Met/Met homozygotes had a higher risk of developing PTSD after extreme stress exposure independent of traumatic load (Kolassa *et al.* 2010). Although not all

of these studies investigated actual cortisol increase, overall our results point in the same direction. *COMT* Met allele carriers (worriers) show an allele dosage dependent stronger cortisol stress response. Intriguingly, these differences between genotype groups were already present in healthy children before the onset of puberty.

COMT is mainly expressed in the PFC (Chen *et al.* 2004; Karoum *et al.* 1994; Matsumoto *et al.* 2003; Tenhunen *et al.* 1993), where it is the most important source for the termination of dopaminergic signalling, a neurotransmitter that has been previously suggested to impact the stress response (Sullivan & Dufresne, 2006). However, it should be noted that COMT also metabolizes norepinephrine, which is an additional potential regulator of stress reactivity (Feldman & Weidenfeld, 2004). The PFC, in turn, has been implicated to be involved in the processing of psychological stressors (Dedovic *et al.* 2009*b*), thus suggesting a mechanism through which *COMT* might exert an influence on peripheral stress parameters. However, the PFC is also involved in cognitive functions and *COMT* has been found to impact working memory and cognitive flexibility (Barnett *et al.* 2007; Bilder *et al.* 2004; Joober *et al.* 2002; Malhotra *et al.* 2002). Since the TSST-C is not only a stressful situation, but also a cognitive challenge, this potentially confounding factor needs to be acknowledged. Hence, the effects of *COMT* on HPA axis reactivity should additionally be investigated with stress paradigms that do not require such a degree of cognitive processing. Furthermore, as Oswald *et al.* (2004) pointed out, *COMT* might also exert its influence on the HPA axis via its actions on the opioid neurotransmission.

Since the dopaminergic system is further modulated by BDNF (Horger *et al.* 1999; Hyman *et al.* 1991; Narita *et al.* 2003; Spina *et al.* 1992), we included the *BDNF* Val^{66}Met SNP in our analysis. While there was no significant effect of *BDNF* on cortisol levels across the different points in time, regression analysis revealed a stronger cortisol increase in Met allele carriers. The latter finding is in line with other studies reporting stronger cortisol responses in *BDNF* Met allele carriers (Colzato *et al.* 2011; Shalev *et al.* 2009; Vinberg *et al.* 2009). However, there was no *COMT* × *BDNF* interaction. In addition to the potentially moderating role of BDNF, a recent rodent study demonstrated that the well-known link between HPA axis activity and a further neurotransmitter – serotonin (5-HT) – was modulated by 5-HT$_{2C}$ receptors (Heisler *et al.* 2007). 5-HT$_{2C}$ was found to be expressed in CRH-containing neurons in the PVN and affected CRH regulation and expression. Since interactions between the serotonergic and dopaminergic system are well documented (review in di Giovanni *et al.* 2010), this raises the likelihood of an additional possible route of influence for COMT through CRH regulation via 5-HT$_{2C}$. Recently, *COMT* Val^{158}Met × *5-HT*$_{2C}$ – 759 C/T interactions were reported on BMI, fat BMI, waist circumference and cholesterol levels (Kring *et al.* 2009), pointing to joint influence in metabolic disorders, which, in turn, are linked to mood disorders and stress regulation.

While the genetic effects are in line with existing findings, our results concerning SLEs might appear contradictory to previous results. SLEs have been indicated as a risk factor for neurospsychiatric outcomes (Paykel, 2003). Thus, one might expect a positive association between stressful events and the cortisol stress response. However, there are two additional points to consider. First, dysfunctional HPA activity does not necessarily manifest itself in an exaggerated cortisol secretion. Blunted cortisol responses have, for instance, been reported in patients with panic disorder (Petrowski *et al.* 2010) or depression (Burke *et al.* 2005*a*, *b*). Second, and more importantly, reported SLEs were mild to moderate. There were no maltreated children in our sample. Rather, a positive selection is to be expected since it seems highly unlikely that abusive parents would participate in a study aiming to investigate SLEs in their children's life – events about which they would be questioned in an interview. In addition, participants were preselected to be healthy. Also, although some parents reported financial problems, these were at the lower end of the range. Notably, the SLE effect was apparently mainly driven by death of significant others and, to a lesser degree, by family problems.

In sum, our sample consisted of very healthy children from overall functioning social backgrounds, who, although differing with regard to past stressors, certainly did not experience the entire range of possible SLEs. This restriction in variability might have obscured possible associations, including gene × environment interactions. Clearly, replications in more heterogeneous samples are warranted. Furthermore, longitudinal studies are needed to shed light on the dynamic processing of stressful events and on their influence on shaping future stress responses and the development of coping strategies. Based on the mild to moderate SLE burden, we interpret our finding of an inverse relationship between SLEs and the cortisol response as a sign of resilience. Children who had experienced more stressors, but were apparently able to handle them well enough since there were no signs of impaired health or social functioning, appeared to be better prepared to deal with a new

stressor (TSST-C). Findings in animal and human studies suggest that mild to moderate stressors during development might be beneficial since they may lead to a more adaptive phenotype, including diminished HPA axis activation after acute stress exposure (for a review, see Lyons & Parker, 2007). Thus, the relationship between SLEs and stress-related outcomes might follow a U-shaped profile (e.g. Macrì & Würbel, 2006). However, stress reactivity does not solely depend on encountered SLEs but also on individual genotype composition. This notion is supported by reports of subgroups that have been found to 'benefit' even from traumatic events, showing what has been described as post-traumatic growth (Tedeschi & Calhoun, 2004) while others might develop PTSD. Although we did not observe a *COMT* × SLE interaction, which might be due to the aforementioned restricted SLE variance, such an effect was found at the other end of the SLE spectrum. The development of PTSD in survivors of the Rwandan Genocide was moderated by *COMT* (Kolassa *et al.* 2010).

There are several limitations to our study. The sample size was comparatively small for a genetic association study. Furthermore, the relatively strict selection procedure may have reduced representativeness and may have also led to a restriction of variability of the cortisol stress response. This restriction of variability might, in turn, have obscured possible associations, which, however, could have also been obscured by additional confounding factors, such as the use of medication or physical and mental disorders. Thus, we chose to exclude known or likely confounding factors and take the risk that some effects might not become significant due to restriction of variability rather than the opposite. Clearly, however, future studies should examine whether the present results hold true in more heterogeneous populations.

Taken together, our findings provide further evidence that the cortisol stress response is sensitive for genetic variation in *COMT*. The Met allele, which leads to enhanced catecholinergic activity, resulted in a stronger response. In addition, a less pronounced cortisol increase was related to more (mild to moderate) stressful events, probably representing increased resilience and an enhanced ability to deal with new stressors. However, the exact underlying biological pathways by which *COMT* Val^{158}Met and SLEs impact the stress response need to be investigated further.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (KI 537/20-1, 20-3) and SFB 581, KFO 125 and SFB TTR 58 to K.P.L. We thank Nicole Steigerwald and Nicole Döring for their excellent technical assistance in DNA sample processing and genotyping and Gabriele Arnold and Sarah Brand for conducting cortisol analyses.

Statement of Interest

None.

References

Aberg E, Fandino-Losada A, Sjöholm LK, Forsell Y, *et al.* (2011). The functional Val158Met polymorphism in catechol-O-methyltransferase (COMT) is associated with depression and motivation in men from a Swedish population-based study. *Journal of Affective Disorders* **129**, 158–166.

Alexander N, Osinsky R, Mueller E, Schmitz A, *et al.* (2011). Genetic variants within the dopaminergic system interact to modulate endocrine stress reactivity and recovery. *Behavioural Brain Research* **216**, 53–58.

Armbruster D, Mueller A, Strobel A, Lesch KP, *et al.* (2011). Variation in genes involved in dopamine clearance influence the startle response in older adults. *Journal of Neural Transmission* **118**, 1281–1292.

Arnsten AF, Goldman-Rakic PS (1998). Noise stress impairs prefrontal cortical cognitive function in monkeys: evidence for a hyperdopaminergic mechanism. *Archives of General Psychiatry* **55**, 362–368.

Axinn WG, Pearce LD, Ghimire D (1999). Innovations in life history calendar applications. *Social Science Research* **28**, 243–264.

Barnett JH, Jones PB, Robbins TW, Muller U (2007). Effects of the catechol-O-methyltransferase Val158Met polymorphism on executive function: a meta-analysis of the Wisconsin Card Sort Test in schizophrenia and healthy controls. *Molecular Psychiatry* **12**, 502–509.

Bilder RM, Volavka J, Lachman HM, Grace AA (2004). The catechol-O-methyltransferase polymorphism: relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. *Neuropsychopharmacology* **29**, 1943–1961.

Blasi G, Mattay VS, Bertolino A, Elvevag B, *et al.* (2005). Effect of catechol-O-methyltransferase val158met genotype on attentional control. *Journal of Neuroscience* **25**, 5038–5045.

Brennan PA, Hammen C, Sylvers P, Bor W, *et al.* (2011). Interactions between the COMT Val108/158Met polymorphism and maternal prenatal smoking predict aggressive behavior outcomes. *Biological Psychology* **87**, 99–105.

Burke HM, Davis MC, Otte C, Mohr DC (2005*a*). Depression and cortisol responses to psychological stress: a meta-analysis. *Psychoneuroendocrinology* **30**, 846–856.

Burke HM, Fernald LC, Gertler PJ, Adler NE (2005*b*). Depressive symptoms are associated with blunted cortisol

stress responses in very low-income women. *Psychosomatic Medicine* **67**, 211–216.

Buske-Kirschbaum A, Jobst S, Wustmans A, Kirschbaum C, *et al.* (1997). Attenuated free cortisol response to psychosocial stress in children with atopic dermatitis. *Psychosomatic Medicine* **59**, 419–426.

Caspi A, Moffitt TE, Cannon M, McClay J, *et al.* (2005). Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene × environment interaction. *Biological Psychiatry* **57**, 1117–1127.

Caspi A, Moffitt TE, Thornton A, Freedman D, *et al.* (1996). The life history calendar: a research and clinical assessment method for collecting retrospective event-history data. *International Journal of Methods in Psychiatric Research* **6**, 101–114.

Chen J, Lipska BK, Halim N, Ma QD, *et al.* (2004). Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *American Journal of Human Genetics* **75**, 807–821.

Colzato LS, van der Does AJ, Kouwenhoven C, Elzinga BM, *et al.* (2011). BDNF Val(66)Met polymorphism is associated with higher anticipatory cortisol stress response, anxiety, and alcohol consumption in healthy adults. *Psychoneuroendocrinology* **36**, 1562–1569.

Dedovic K, d'Aguiar C, Pruessner JC (2009*a*). What stress does to your brain: a review of neuroimaging studies. *Canadian Journal of Psychiatry* **54**, 6–15.

Dedovic K, Duchesne A, Andrews J, Engert V, *et al.* (2009*b*). The brain and the stress axis: the neural correlates of cortisol regulation in response to stress. *Neuroimage* **47**, 864–871.

di Giovanni G, Esposito E, di Matteo V (2010). Role of serotonin in central dopamine dysfunction. *CNS Neuroscience and Therapeutics* **16**, 179–194.

Drabant EM, Hariri AR, Meyer-Lindenberg A, Munoz KE, *et al.* (2006). Catechol O-methyltransferase val158met genotype and neural mechanisms related to affective arousal and regulation. *Archives of General Psychiatry* **63**, 1396–1406.

Dumontheil I, Roggeman C, Ziermans T, Peyrard-Janvid M, *et al.* (2011). Influence of the COMT genotype on working memory and brain activity changes during development. *Biological Psychiatry* **70**, 222–229.

Egan MF, Goldberg TE, Kolachana BS, Callicott JH, *et al.* (2001). Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proceedings of the National Academy of Sciences USA* **98**, 6917–6922.

Elzinga BM, Molendijk ML, Oude Voshaar RC, Bus BA, *et al.* (2011). The impact of childhood abuse and recent stress on serum brain-derived neurotrophic factor and the moderating role of BDNF Val66Met. *Psychopharmacology* (*Berlin*) **214**, 319–328.

Enoch MA, Xu K, Ferro E, Harris CR, *et al.* (2003). Genetic origins of anxiety in women: a role for a functional catechol-O-methyltransferase polymorphism. *Psychiatric Genetics* **13**, 33–41.

Feldman S, Weidenfeld J (2004). Involvement of endogeneous glutamate in the stimulatory effect of norepinephrine and serotonin on the hypothalamo-pituitary-adrenocortical axis. *Neuroendocrinology* **79**, 43–53.

Freedman D, Thornton A, Camburn D, Alwin D, *et al.* (1988). The life history calendar: a technique for collecting retrospective data. *Sociological Methodology* **18**, 37–68.

Fumagalli F, Molteni R, Racagni G, Riva MA (2007). Stress during development: impact on neuroplasticity and relevance to psychopathology. *Progress in Neurobiology* **81**, 197–217.

Goldberg TE, Egan MF, Gscheidle T, Coppola R, *et al.* (2003). Executive subprocesses in working memory: relationship to catechol-O-methyltransferase Val158Met genotype and schizophrenia. *Archives of General Psychiatry* **60**, 889–896.

Goldman D, Oroszi G, Ducci F (2005). The genetics of addictions: uncovering the genes. *Nature Reviews Genetics* **6**, 521–532.

Heisler LK, Pronchuk N, Nonogaki K, Zhou L, *et al.* (2007). Serotonin activates the hypothalamic-pituitary-adrenal axis via serotonin 2C receptor stimulation. *Journal of Neuroscience* **27**, 6956–6964.

Horger BA, Iyasere CA, Berhow MT, Messer CJ, *et al.* (1999). Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. *Journal of Neuroscience* **19**, 4110–4122.

Hünnerkopf R, Strobel A, Gutknecht L, Brocke B, *et al.* (2007). Interaction between BDNF Val66Met and dopamine transporter gene variation influences anxiety-related traits. *Neuropsychopharmacology* **32**, 2552–2560.

Hyman C, Hofer M, Barde YA, Juhasz M, *et al.* (1991). BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature* **350**, 230–232.

Jabbi M, Kema IP, van der Pompe G, te Meerman GJ, *et al.* (2007). Catechol-o-methyltransferase polymorphism and susceptibility to major depressive disorder modulates psychological stress response. *Psychiatric Genetics* **17**, 183–193.

Jankord R, Herman JP (2008). Limbic regulation of hypothalamo-pituitary-adrenocortical function during acute and chronic stress. *Annals of the New York Academy of Sciences* **1148**, 64–73.

Joober R, Gauthier J, Lal S, Bloom D, *et al.* (2002). Catechol-O-methyltransferase Val-108/158-Met gene variants associated with performance on the Wisconsin Card Sorting Test. *Archives of General Psychiatry* **59**, 662–663.

Karoum F, Chrapusta SJ, Egan MF (1994). 3-Methoxytyramine is the major metabolite of released dopamine in the rat frontal cortex: reassessment of the effects of antipsychotics on the dynamics of dopamine release and metabolism in the frontal cortex, nucleus accumbens, and striatum by a simple two pool model. *Journal of Neurochemistry* **63**, 972–979.

Kaufman J, Plotsky PM, Nemeroff CB, Charney DS (2000). Effects of early adverse experiences on brain structure and function: clinical implications. *Biological Psychiatry* **48**, 778–790.

Kino T, Chrousos GP (2005). Glucocorticoid effects on gene expression. In: Steckler T, Kalin NH, Reul JMHM (Eds.), *Handbook of Stress and the Brain* (pp. 295–311). Amsterdam, London: Elsevier.

Kirschbaum C, Pirke KM, Hellhammer DH (1993). The 'Trier Social Stress Test' – a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* **28**, 76–81.

Kirschbaum C, Wüst S, Faig HG, Hellhammer DH (1992). Heritability of cortisol responses to human corticotropin-releasing hormone, ergometry, and psychological stress in humans. *Journal of Clinical Endocrinology and Metabolism* **75**, 1526–1530.

Kolassa IT, Kolassa S, Ertl V, Papassotiropoulos A, *et al.* (2010). The risk of posttraumatic stress disorder after trauma depends on traumatic load and the catechol-O-methyltransferase Val(158)Met polymorphism. *Biological Psychiatry* **67**, 304–308.

Kring SI, Werge T, Holst C, Toubro S, *et al.* (2009). Polymorphisms of serotonin receptor 2A and 2C genes and COMT in relation to obesity and type 2 diabetes. *PLoS ONE* **4**, e6696.

Lang UE, Bajbouj M, Sander T, Gallinat J (2007). Gender-dependent association of the functional catechol-O-methyltransferase Val158Met genotype with sensation seeking personality trait. *Neuropsychopharmacology* **32**, 1950–1955.

Laucht M, Blomeyer D, Buchmann AF, Treutlein J, *et al.* (2011). Catechol-O-methyltransferase Val(158) Met genotype, parenting practices and adolescent alcohol use: testing the differential susceptibility hypothesis. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, doi:10.1111/j.1469-7610.2011.02408.x, published online 19 April 2011.

Linkowski P, Van Onderbergen A, Kerkhofs M, Bosson D, *et al.* (1993). Twin study of the 24-h cortisol profile: evidence for genetic control of the human circadian clock. *American Journal of Physiology* **264**, E173–E181.

Lyons DM, Parker KJ (2007). Stress inoculation-induced indications of resilience in monkeys. *Journal of Traumatic Stress* **20**, 423–433.

McClearn GE (2006). Contextual genetics. *Trends in Genetics* **22**, 314–319.

Macrì S, Würbel H (2006). Developmental plasticity of HPA and fear responses in rats: a critical review of the maternal mediation hypothesis. *Hormones and Behavior* **50**, 667–680.

Malhotra AK, Kestler LJ, Mazzanti C, Bates JA, *et al.* (2002). A functional polymorphism in the COMT gene and performance on a test of prefrontal cognition. *American Journal of Psychiatry* **159**, 652–654.

Matsumoto M, Weickert CS, Akil M, Lipska BK, *et al.* (2003). Catechol O-methyltransferase mRNA expression in human and rat brain: evidence for a role in cortical neuronal function. *Neuroscience* **116**, 127–137.

Mier D, Kirsch P, Meyer-Lindenberg A (2010). Neural substrates of pleiotropic action of genetic variation in COMT: a meta-analysis. *Molecular Psychiatry* **15**, 918–927.

Montag C, Buckholtz JW, Hartmann P, Merz M, *et al.* (2008). COMT genetic variation affects fear processing: psychophysiological evidence. *Behavioural Neuroscience* **122**, 901–909.

Murakami S, Imbe H, Morikawa Y, Kubo C, *et al.* (2005). Chronic stress, as well as acute stress, reduces BDNF mRNA expression in the rat hippocampus but less robustly. *Neuroscience Research* **53**, 129–139.

Murphy BL, Arnsten AF, Goldman-Rakic PS, Roth RH (1996). Increased dopamine turnover in the prefrontal cortex impairs spatial working memory performance in rats and monkeys. *Proceedings of the National Academy of Sciences USA* **93**, 1325–1329.

Narita M, Aoki K, Takagi M, Yajima Y, *et al.* (2003). Implication of brain-derived neurotrophic factor in the release of dopamine and dopamine-related behaviors induced by methamphetamine. *Neuroscience* **119**, 767–775.

Olsson CA, Anney RJ, Lotfi-Miri M, Byrnes GB, *et al.* (2005). Association between the COMT Val158Met polymorphism and propensity to anxiety in an Australian population-based longitudinal study of adolescent health. *Psychiatric Genetics* **15**, 109–115.

Oswald LM, McCaul M, Choi L, Yang X, *et al.* (2004). Catechol-O-methyltransferase polymorphism alters hypothalamic-pituitary-adrenal axis responses to naloxone: a preliminary report. *Biological Psychiatry* **55**, 102–105.

Paykel ES (2003). Life events and affective disorders. *Acta Psychiatrica Scandinavica* (Supplementum) 108, 61–66.

Perroud N, Jaussent I, Guillaume S, Bellivier F, *et al.* (2010). COMT but not serotonin-related genes modulates the influence of childhood abuse on anger traits. *Genes, Brain and Behavior* **9**, 193–202.

Petrowski K, Herold U, Joraschky P, Wittchen HU, *et al.* (2010). A striking pattern of cortisol non-responsiveness to psychosocial stress in patients with panic disorder with concurrent normal cortisol awakening responses. *Psychoneuroendocrinology* **35**, 414–421.

Rasch B, Spalek K, Buholzer S, Luechinger R, *et al.* (2010). Aversive stimuli lead to differential amygdala activation and connectivity patterns depending on catechol-O-methyltransferase Val158Met genotype. *Neuroimage* **52**, 1712–1719.

Reuter M, Hennig J (2005). Association of the functional catechol-O-methyltransferase VALI58MET polymorphism with the personality trait of extraversion. *Neuroreport* **16**, 1135–1138.

Roceri M, Hendriks W, Racagni G, Ellenbroek BA, *et al.* (2002). Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. *Molecular Psychiatry* **7**, 609–616.

Schule C, Zill P, Baghai TC, Eser D, *et al.* (2006). Brain-derived neurotrophic factor Val66Met polymorphism and dexamethasone/CRH test results in

depressed patients. *Psychoneuroendocrinology* **31**, 1019–1025.

Shalev I, Lerer E, Israel S, Uzefovsky F, *et al.* (2009). BDNF Val66Met polymorphism is associated with HPA axis reactivity to psychological stress characterized by genotype and gender interactions. *Psychoneuroendocrinology* **34**, 382–388.

Smolka MN, Schumann G, Wrase J, Grusser SM, *et al.* (2005). Catechol-O-methyltransferase val158met genotype affects processing of emotional stimuli in the amygdala and prefrontal cortex. *Journal of Neuroscience* **25**, 836–842.

Song L, Che W, Min-Wei W, Murakami Y, *et al.* (2006). Impairment of the spatial learning and memory induced by learned helplessness and chronic mild stress. *Pharmacology, Biochemistry and Behavior* **83**, 186–193.

Spina MB, Squinto SP, Miller J, Lindsay RM, *et al.* (1992). Brain-derived neurotrophic factor protects dopamine neurons against 6-hydroxydopamine and N-methyl-4-phenylpyridinium ion toxicity: involvement of the glutathione system. *Journal of Neurochemistry* **59**, 99–106.

Stein DJ, Newman TK, Savitz J, Ramesar R (2006). Warriors *vs.* worriers: the role of COMT gene variants. *CNS Spectrums* **11**, 745–748.

Stein MB, Fallin MD, Schork NJ, Gelernter J (2005). COMT polymorphisms and anxiety-related personality traits. *Neuropsychopharmacology* **30**, 2092–2102.

Sullivan RM, Dufresne MM (2006). Mesocortical dopamine and HPA axis regulation: role of laterality and early environment. *Brain Research* **1076**, 49–59.

Tarullo AR, Gunnar MR (2006). Child maltreatment and the developing HPA axis. *Hormones and Behavior* **50**, 632–639.

Tedeschi RG, Calhoun LG (2004). Posttraumatic growth: conceptual foundations and empirical evidence. *Psychological Inquiry* **15**, 1–18.

Tenhunen J, Salminen M, Jalanko A, Ukkonen S, *et al.* (1993). Structure of the rat catechol-O-methyltransferase gene: separate promoters are used to produce mRNAs for soluble and membrane-bound forms of the enzyme. *DNA and Cell Biology* **12**, 253–263.

Tunbridge EM, Bannerman DM, Sharp T, Harrison PJ (2004). Catechol-o-methyltransferase inhibition improves set-shifting performance and elevates stimulated dopamine release in the rat prefrontal cortex. *Journal of Neuroscience* **24**, 5331–5335.

Vinberg M, Trajkovska V, Bennike B, Knorr U, *et al.* (2009). The BDNF Val66Met polymorphism: relation to familiar risk of affective disorder, BDNF levels and salivary cortisol. *Psychoneuroendocrinology* **34**, 1380–1389.

Walder DJ, Trotman HD, Cubells JF, Brasfield J, *et al.* (2010). Catechol-O-methyltransferase modulation of cortisol secretion in psychiatrically at-risk and healthy adolescents. *Psychiatric Genetics* **20**, 166–170.

Weaver IC (2007). Epigenetic programming by maternal behavior and pharmacological intervention. Nature *vs.* nurture: let's call the whole thing off. *Epigenetics* **2**, 22–28.

Weinshilboum RM, Otterness DM, Szumlanski CL (1999). Methylation pharmacogenetics: catechol O-methyltransferase, thiopurine methyltransferase, and histamine N-methyltransferase. *Annual Review of Pharmacology and Toxicology* **39**, 19–52.

West SG, Aiken LS, Krull JL (1996). Experimental personality designs: analyzing categorical by continuous variable interactions. *Journal of Personality* **64**, 1–48.

Williams LM, Gatt JM, Grieve SM, Dobson-Stone C, *et al.* (2010). COMT Val(108/158)Met polymorphism effects on emotional brain function and negativity bias. *Neuroimage* **53**, 918–925.

Winterer G, Musso F, Vucurevic G, Stoeter P, *et al.* (2006). COMT genotype predicts BOLD signal and noise characteristics in prefrontal circuits. *Neuroimage* **32**, 1722–1732.

Woo JM, Yoon KS, Choi YH, Oh KS, *et al.* (2004). The association between panic disorder and the L/L genotype of catechol-O-methyltransferase. *Journal of Psychiatric Research* **38**, 365–370.

Wray NR, James MR, Dumenil T, Handoko HY, *et al.* (2008). Association study of candidate variants of COMT with neuroticism, anxiety and depression. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* **147B**, 1314–1318.

Zubieta JK, Heitzeg MM, Smith YR, Bueller JA, *et al.* (2003). COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. *Science* **299**, 1240–1243.