Predicting cortisol stress responses in older individuals: Influence of serotonin receptor 1A gene (HTR1A) and stressful life events

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ABSTRACT

Considerable variability in the activity of the hypothalamus–pituitary–adrenal (HPA) axis in response to stress has been found in quantitative genetic studies investigating healthy individuals suggesting that at least part of this variance is due to genetic factors. Since the HPA axis is regulated by a neuronal network including amygdala, hippocampus, prefrontal cortex as well as brainstem circuits, the investigation of candidate genes that impact neurotransmitter systems related to these brain regions might further elucidate the genetic underpinnings of the stress response. However, aside from genetic risk factors, past stressful life events might also result in long-term adjustments of HPA axis reactivity. Here, we investigated the effects of the −1019 G/C polymorphism in the HTR1A gene encoding the serotonin (5-HT) receptor 1A (5-HT1A) and stressful life events experienced during childhood and adolescence on changes in cortisol levels in response to the Trier Social Stress Test (TSST) in a sample of healthy older adults (N = 97). Regression analyses revealed a significant effect of HTR1A genotype with the G allele being associated with a less pronounced stress response. In addition, an inverse relationship between past stressful life events and cortisol release but no gene × environment interaction was detected. The results further underscore the crucial role of functional serotonergic genetic variation as well as stressful events during critical stages of development on the acute stress response later in life.

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Introduction

Encountering stressful situations leads to a wide variety of psychological and physiological changes including activation of the hypothalamic–pituitary–adrenal (HPA) axis. In short, stress results in the release of corticotropin releasing hormone (CRH) from the paraventricular nucleus (PVN) of the hypothalamus with a subsequent stimulation of adrenocorticotropic (ACTH) and cortisol secretion in humans. Cortisol as the major stress hormone influences numerous physiological systems, including CNS function, metabolism, cardiovascular function, immune system, muscle tissue, and bones (Kino and Chrousos, 2005). The degree of change in cortisol levels in response to stress varies widely even in healthy individuals with genetic as well as environmental factors as likely contributors (Kirschbaum and Hellhammer, 1999). Since dysregulation of HPA axis activity has been related to impairing conditions like depression, anxiety and several other disorders (for a review see e.g., Chrousos, 2009; Handwerger, 2009; Pariante and Lightman, 2008) which are associated with considerable suffering as well as elevated health care costs, it is vital to investigate potential genetic and environmental factors in greater detail. HPA axis activity is further modulated by additional neural circuits, including the brainstem, the amygdala, the hippocampus and the medial prefrontal cortex (PFC; Dedovic et al., 2009a; Jankord and Herman, 2008), and thus, one promising research strategy to further elucidate its genetic underpinnings might be the investigation of candidate genes that impact neurotransmitter systems related to these brain regions.

Serotonin (5-HT) has been implicated in more behavioral, physiological and pathological mechanisms than any other brain neurotransmitter (Azmitia, 2007) and differences in serotonergic function based on genetic variation and their association with behavioral outcomes have been investigated in numerous studies in healthy volunteers and clinical samples. Regarding the cortisol stress response, 5-HT has been found to modulate HPA axis reactivity (Holmes, 2008; Lanfumey et al., 2008) with serotoninergic neurons from the raphe nuclei projecting to the PVN of the hypothalamus (Herman et al., 2005). In addition, serotonergic neurons innervate the amygdala (Jacobs and Azmitia, 1992) which in turn also has
connections to the PVN via the bed nucleus of the stria terminalis (BNST) (Davis and Whalen, 2001; Jankord and Herman, 2008). The relationship between 5-HT and the HPA axis is not one-directional; administration of glucocorticoids or activation of the HPA axis through stress exposure has been found to affect the serotonergic system as well (Leonard, 2005), with CRH-immunoreactive cell fibers in the rostral and caudal raphe nuclei being part of the neuroanatomical basis for HPA axis influence on 5-HT neurotransmission (Linthorst, 2005). Thus, HPA axis and the serotonergic system form a multifaceted network (Porter et al., 2004). Here, we focus on the effects of variation in the gene encoding for the 5-HT1A receptor (HTR1A) as one of the essential regulators of 5-HT function on the cortisol stress response.

5-HT1A is a G-protein coupled receptor which in the raphe nuclei functions as an autoreceptor providing negative feedback to the afferent neuron. 5-HT1A is also expressed postsynaptically in the hippocampus, entorhinal cortex, septum, periaqueductal gray, frontal cortex, and amygdala where they inhibit neuronal activity by hyperpolarization (Albert and Lemonde, 2004; Pineyro and Blier, 1999). Altered 5-HT1A function has been suggested to contribute to the pathogenesis of anxiety and depressive disorders (Nash et al., 2008; Neumeister et al., 2004) and human as well as animal studies have linked 5-HT1A to differences in emotional regulation: mice with higher aggression scores showed a heightened expression of 5-HT1A receptors in the dorsal hippocampus compared to less aggressive mice (Korte et al., 1996) while 5-HT1A knock-out mice display increased anxiety-related behaviors compared to normal wild-type controls although they also show decreased behavioral despair in response to stress (Gross and Hen, 2004; Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998) which has been referred to as “antidepressed” phenotype (Richardson-Jones et al., 2010). Recently, mice strains in which only 5-HT1A autoreceptor (but not postsynaptic receptor) function was altered were found to show changes of physiological stress responses, behavioral despair, and response to antidepressants but no differences in anxiety-like behavior (Richardson-Jones et al., 2010). Regarding the link between 5-HT1A and the HPA axis, administration of a 5-HT1A antagonist before exposing rats to single-prolonged stress resulted in decreased CRH and glucocorticoid receptor mRNA and protein levels (Wang et al., 2009). In turn, adrenalectomy in rats leads to anatomically specific decreases in serotonergic metabolic indices and to a significant increase in 5-HT1A receptor binding and mRNA levels in the hippocampus (Grino et al., 1987). Consistently, 5-HT1A receptor mRNA levels and 5-HT1A-binding were reduced in the rat hippocampus after 2 weeks of chronic stress (Lopez et al., 1998).

In humans, HTR1A is located on chromosome 5q12.3 and contains in its transcriptional control region a functional C/G single nucleotide polymorphism (SNP) at position −1019 (rs6295) with the G variant preventing binding of regulatory proteins resulting in an increased HTR1A gene expression and 5-HT1A-mediated serotonergic neurotransmission (Albert and Lemonde, 2004; Lemonde et al., 2003). Consistently, 5-HT1A autoreceptor density was reported to be increased in G allele carriers in a positron emission tomography (PET) study (Parsey et al., 2006b) although not in an earlier PET study (David et al., 2005). In vitro findings point to increased expression of 5-HT1A autoreceptors but decreased 5-HT1A expression in postsynaptic receptors in G allele carriers (Czesak et al., 2006). The HTR1A G allele has been associated with major depression (Anttila et al., 2007; Kraus et al., 2007; Lemonde et al., 2003; Neff et al., 2009; Parsey et al., 2006a), panic attacks (Huang et al., 2004) and panic disorder with agoraphobia (Rothe et al., 2004) and anxiety- and depression-related personality traits (i.e., neuroticism and harm avoidance; Strobel et al., 2003) although there are also inconsistent findings (Koller et al., 2006). Recently, neuroimaging studies reported a link between decreased amygdala reactivity and increased 5-HT1A autoreceptor expression and the presence of the HTR1A G allele, respectively (Fakra et al., 2009; Fisher et al., 2006). However, the resulting consequences on downstream effector systems of the amygdala such as the HPA axis need to be investigated as well since such intermediate phenotypes can further narrow the gap between brain activation and behavioral variables.

Similarly, there is evidence on the potential modulation of the cortisol stress response by stressful life events (SLEs), which have been found to contribute substantially to less favorable neuropsychiatric outcomes (Paykel, 2003) like depression or anxiety. Particularly, SLEs occurring in critical early stages of life have been found to result in persisting negative effects over the course of life in human as well as in animal studies: in rats, an early adverse social environment (i.e., low maternal care) has been reported to increase stress responses in adult rats via epigenetic processes (overview in Weaver, 2007). In humans, the crucial role of past events particularly during early development has been reported in several studies (e.g., Fumagalli et al., 2007; Kaufman et al., 2000) as have been gene × environment interactions (e.g., Canli and Lesch, 2007; Caspi et al., 2010; Kaufman et al., 2004; Lesch, 2004), the latter, however, mainly focused on the role of genetic variation in 5-HT transporter function. Thus, in the present study we investigated the independent and joint effects of HTR1A −1019 C/G genotype and SLEs (experienced during childhood and adolescence) on the cortisol stress response in an acute social stress paradigm in a sample of older adults which enabled us to address an additional question: whether the potentially negative effects of early SLEs persist throughout the life span into late adulthood or whether they only exert a transient influence that will eventually fade out.

Methods

Participants

All of our participants were of German/Western European ancestry and originally consisted of 62 female and 40 male older adults. Of these, 97 participants were successfully genotyped for the HTR1A −1019 SNP leaving 60 female and 37 male older participants for the final sample (mean age 61.15 years, SD = 2.67, range 54–68 years). All participants were non-smokers and reported to be in good health. They were screened for psychiatric or neurological disorders or treatment before participation. In addition, during the course of the interview regarding critical life events, medical problems including psychiatric conditions were documented. Participants were informed about the aims of the study, gave written informed consent and received monetary compensation for participation. The study design was approved by the Ethics Committee of the German Psychological Association.

TSST psychosocial stress protocol

The Trier Social Stress Test (TSST; Kirschbaum et al., 1993) was employed for the induction of psychosocial stress. This standardized laboratory stressor consists of a free speech and a mental arithmetic task in front of an audience and has been shown to result in significant endocrine, cardiovascular, immune, and subjective responses (Kudielka et al., 2007). Including an introduction and a preparation phase, the total procedure takes approximately 15 min. The TSST has been found to elicit the strongest and most reliable cortisol responses to laboratory stress compared with other protocols (Dickerson and Kemeny, 2004).

Cortisol analysis

Salivary cortisol samples were obtained using “Salivettes” (Sarstedt; Rommelsdorf, Germany) and kept at −20 °C until analysis. Samples were collected for determination of the biologically active “free” fraction of cortisol repeatedly before onset of the stress sessions as well as 2, 10,
20, and 30 min after cessation of stress, respectively. Salivary cortisol samples were prepared for biochemical analysis by centrifuging at 3000 rpm for 5 min, which resulted in a clear supernatant of low viscosity. Salivary free cortisol concentrations were determined employing a chemiluminescence immunoassay (CLIA) with high sensitivity of 0.16 ng/ml (IBL; Hamburg, Germany). Intra- and inter-assay coefficients of variation were below 8%.

Statistical analysis

All analyses were performed using SPSS for Windows 16.0 (SPSS Inc., Chicago, IL, USA).

Firstly, in order to determine whether the TSST 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. HTR1A genotype was entered as an independent between subjects variable for a first assessment of group differences between the three different genotypes (G/G vs. G/C vs. C/C) regarding the cortisol stress response. Following, to analyze the impact of HTR1A genotype further as well as the influence of past SLEs, a difference score was computed between cortisol concentrations 20 min after cessation of stress (when cortisol levels had reached its peak) and immediately before the TSST. Since stressful life events are continuously distributed, a linear regression analysis was performed with HTR1A and SLEs during childhood and adolescence as predictors and the cortisol difference score as the dependent variable. In order to assess a possible gene–environment interaction a moderated regression was then conducted. Beforehand, SLE as a continuous predictor was centered and HTR1A genotype as a categorical predictor was recoded as two variables according to the recommendations by West et al. (1996). The products of these transformed independent variables were calculated and entered (West et al., 1996). Furthermore, an additional regression analysis was performed with HTR1A genotype and the ten SLE subcategories as predictors to investigate possible differential effects of the various types of SLEs (e.g., severe illness, death of significant others, physical maltreatment, etc.).

Results

Genotype frequencies

The percentages of the HTR1A genotypes were 20.6% (n = 20) for C/C, 56.7% (n = 55) for C/G, and 22.6% (n = 22) for G/G. The genotypes were in Hardy-Weinberg equilibrium (P = 0.185). The genetic groups did not differ with regard to age (one-way ANOVA, P = 0.368), sex (χ²-test, P = 0.636) or reported stressful life events in any of the developmental periods (one-way ANOVAs, all P ≥ 0.304).

Stressful life events

The average number of reported SLEs in the period from 0 to 15 years of age was 4.11 (SD = 2.92; range, 0–15). It should be noted that a part of the sample reported no SLE at all in this period (8.2%) and a substantial number of participants experienced only one (10.3%) or two SLEs (14.4%). Thus, about a third of our sample experienced relatively few SLEs during childhood and adolescence according to their self-report.

Impact of HTR1A and stressful life events on cortisol response

An ANOVA revealed a significant time effect indicating that the TSST had led to significant changes in cortisol levels (F = 91.98, P < 0.001, η² = 0.397). In addition, there was a main effect of HTR1A genotype (F = 3.82, P = 0.025, η² = 0.075) with C/C homozygotes showing the strongest and G/G homozygotes, the smallest cortisol response while heterozygotes fell in between (Fig. 1). Contrast analyses revealed a significant difference between C/C and G/G (P = 0.012) as well as between C/G and G/G (P = 0.021) while the difference between C/C and C/G was not significant (P = 0.443). Linear regression (R = 0.309) also showed a significant effect for HTR1A genotype (P = 0.032) with a smaller cortisol increase after the TSST in G allele carriers (Beta = −0.214; R² = 0.050) (Fig. 2). The average cortisol increase was approximately twice the size in C/C compared to G/G homozygotes (12.054 nmol/l vs. 5.815 nmol/l;
This effect was not due to baseline differences since the three genotype groups did not differ in cortisol levels before the TSST (ANOVA; \( P = 0.212 \)).

Furthermore, there was also a significant effect for stressful life events experienced during childhood and adolescence: the more SLEs had been reported the smaller was the cortisol increase (\( P = 0.034; \) \( \beta = -0.212; \) \( R^2 = 0.0497 \); Fig. 3). Again, this effect was not due to baseline differences since there was no influence of SLEs on cortisol levels before the TSST (linear regression; \( P = 0.879 \)). Moderated multiple regression revealed no interaction effect between SLEs and HTR1A genotype (all \( P \geq 0.271 \)).

Regarding the differential effects of various types of life events there was a significant effect for physical abuse (during childhood and adolescence; \( P = 0.036; \) \( \beta = -0.277; \) \( R^2 = 0.0655 \)). In addition, problem behavior of significant others also had some predictive value although this effect showed only a tendency towards significance (\( P = 0.098; \) \( \beta = -0.176; \) \( R^2 = 0.0243 \)). The other eight SLE subcategories did not predict cortisol stress response (all \( P \geq 0.332 \)).

### Discussion

Our investigation of genetic and environmental underpinnings of the cortisol stress response in older adults revealed significant effects for HTR1A −1019 C/G genotype and SLEs experienced during childhood and adolescence, but no interaction between the two factors. Notably, the SLE effect was mainly driven by physical abuse and to a lesser degree by problem behavior of significant others while other subcategories such as severe illness of self or others, socio-economic problems or relationship problems did not predict the cortisol stress response. Sexual abuse as another highly likely predictor had also no effect although it should be noted that it was present only in very few individuals in our sample. Intriguingly, the presence of the HTR1A G allele and the occurrence of more SLEs were both associated with a less pronounced cortisol increase after the TSST (Figs. 2 and 3). These effects were not due to differences in cortisol baseline levels. At a first glance these findings might appear contradictory since both the HTR1A G allele and early SLEs have been identified as risk factors for neuropsychiatric disorders (for a review see Le Francois et al., 2008; Lesch and Gutknecht, 2004). Early adverse events increase the risk for developing mental disorders (Paykel, 2003) and have also been linked to increased stress responses (Weaver, 2007). For instance, patients with social anxiety disorder with a history of childhood abuse showed an increased cortisol stress response after the TSST compared to patients without childhood abuse or healthy controls (Elzinga et al., 2010). Thus, a larger cortisol response might be expected in groups with more risk factors.

However, potentially maladaptive stress responses do not necessarily manifest themselves in an exaggerated cortisol secretion. A hypo-reactive HPA axis has been suggested to be the end result of prolonged experiences of stressful events together with an initial hyperactivity of the HPA axis (Fries et al., 2005). Reduced HPA axis activity in response to acute stressors has been observed in patients with different stress-related disorders, most prominently with depression (Burke et al., 2005a; Burke et al., 2005b) where it is accompanied by impaired recovery. Recently, patients with panic disorder were also found to display a clearly absent increase in cortisol secretion in response to the TSST although the heart rate response was similar to a group of healthy controls (Petrowski et al., 2010). Since
cortisol facilitates many physiological changes that in the short term help the organism to deal with a stressor at hand (McEwen, 1998; Steckler et al., 2005), a blunted cortisol response might constitute a less effective reaction or even maladaptive response. Nonetheless, despite exhibiting a significantly reduced stress response, the cortisol increase was not completely absent in G/G homozygotes since they showed an average increase of 5.815 nmol/l in response to the TSST—as for instance opposed to the patients with panic disorder who showed a cortisol increase of about 0 nmol/l in the study of Petrowski et al. (2010). Therefore, an alternative explanation should be taken into account: all our participants were preselected to be free of major physical and psychological problems (such as for instance symptoms of depression or elevated anxiety)—no matter how many stressful life events they might have encountered in the past. Thus, one might speculate whether the experience of aversive stressful events might have led to an increased resilience in these individuals who in the aftermath did not develop anxiety- or depression-related symptoms or, alternatively, might have happened to individuals who were resilient to begin with. Not everyone develops maladaptive responses after the experience of aversive experiences. Furthermore, there are also reports of subgroups which “benefit” from such events in that they show what has been described as posttraumatic growth (e.g., Tedeschi and Calhoun, 2004) and which might lead to increased resilience regarding later stressors. This might explain the results in our sample: at least in the subgroup of individuals who reported a comparatively large number of SLEs we might have had a majority of particularly resilient participants since individuals whose experience of many SLEs led to psychological or physiological problems were not included in the study. Thus, the less pronounced cortisol increase in healthy individuals with more past SLEs might be interpreted as a sign of resilience rather than dysfunction.

Similar considerations apply to carriers of the HTR1A G allele, which has been suggested to be a risk factor for affective disorders (Anttila et al., 2007; Huang et al., 2004; Kraus et al., 2007; Lemonde et al., 2003; Neff et al., 2009; Rothe et al., 2004). Since only healthy individuals participated, particularly the subgroup of G allele carriers might have consisted of otherwise rather resilient individuals despite the presence of the HTR1A risk allele. Since complex traits are influenced by a multitude of genetic variations it is possible that these individuals carried more favorable alleles at other genetic loci which counter-balanced effects of the HTR1A G allele. In addition, findings in animal studies showed that mice with higher 5-HT1A autoreceptor function also displayed a blunted physiological response to acute stress (Richardson-Jones et al., 2010) which is in line with our finding since the human HTR1A G allele also results in increased 5-HT1A autoreceptor expression (Czesak et al., 2006). Nonetheless, these mice also showed increased behavioral despair in response to the tail suspension and the forced swim test and no response to a challenge with the antidepressant (Czesak et al., 2006) which in turn has been linked to reduced amygdala activity in response to negative stimuli (Fakra et al., 2009; Fisher et al., 2006). Subsequently, such decreased amygdala reactivity might also contribute to a reduced HPA axis activity in response to stress, albeit a direct influence via brainstem-PVN-connections can also not be ruled out. However, the TSST induces psychosocial stress which may involve hippocampal and prefrontal regions more heavily than physical stressors which may predominantly involve brainstem nuclei and the amygdala (Dedovic et al., 2009b; Puressner et al., 2008; Puressner et al., 2010). Nonetheless, since the amygdala has extensive connections with the hippocampus and PFC as well as with the brainstem (Davis and Whalen, 2001; Jankord and Herman, 2008) it has been suggested that the amygdala is also involved in the processing of psychological stress (Dedovic et al., 2009b). The relationship between HPA axis and serotonergic signaling is highly complex since not only does 5-HTTurnover affect HPA axis activity but HPA axis activity affects the serotonergic system as well (Chauloff et al., 1999; Leonard, 2005; Porter et al., 2004), with CRH-immunoreactive cell fibers in the raphe nuclei forming in part the neuroanatomical basis for HPA axis influence on serotonergic neurotransmission (Linthorst, 2005). Regarding 5-HT receptors, increased levels of corticosteroids during acute stress have been found to increase 5-HT1A mRNA and binding while 2 weeks of chronic stress (Lopez et al., 1998) as well as hypercortisolemia (McAllister-Williams et al., 1998) have been reported to reduce 5-HT1A receptor function. Consistently, adrenalectomy which results in the removal of circulating corticosteroids has been found to significantly increase 5-HT1A receptor binding and mRNA in the hippocampus (Grino et al., 1987). In our sample, SLEs experienced during childhood and adolescence were associated with a lesser cortisol increase in later adulthood. However, at the time of their occurrence these SLEs probably resulted in a considerable cortisol secretion which might have additionally resulted in changes in serotonergic functioning. Thus, long-term changes in HPA axis activity based on past stressful events might result in changes in the responsiveness of the serotonergic systems to acute stress as well (Linthorst, 2005), adding to and/or interacting with already existing differences in neurotransmitter function based on genetic factors such as HTR1A — 1019 G/C. To entangle these mechanisms longitudinal studies are necessary.

There are several limitations to our study that need to be acknowledged. The sample size was quite small for a genetic association study. Furthermore, strict inclusion criteria may have reduced the representativeness of our sample and the variability of the cortisol response. This restriction of variability might have in turn obscured possible associations, which, however, could have been also obscured by additional confounding factors such as smoking, 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 reach significance due to restriction of variability rather than the opposite. It is also important to note that our sample of healthy subjects might have experienced less stressful life events than individuals of comparable age with health problems. More importantly, it is not entirely certain that the smaller cortisol increase in HTR1A risk allele carriers and participants with more past SLEs does indeed signify greater resilience in general since our data were obtained in an exceptionally healthy older sample. Particularly, since there are to date no normal values regarding the cortisol increase after the TSST available, the findings should be interpreted carefully. One clear implication is the need for replication in larger heterogenous samples which would allow contrasting the cortisol response in healthy vs. impaired individuals carrying the same risk alleles and having experienced similar amounts of past aversive events. Should the same pattern of a blunted response or even a non-response also emerge in individuals with health problems then this
would support the notion of a dysfunctional HPA axis reactivity in otherwise healthy participants. Should, however, individuals with health impairments show the opposite pattern (i.e., an exaggerated response) then our current findings would be a strong indicator of resilience. In addition, as noted above, longitudinal studies are necessary to follow the developmental paths after the experience of stressful events over the course of life.

In conclusion, our results further underscore the crucial role of serotonergic genetic variation as well as past stressful life events on stress reactivity. SLEs which occurred decades before our stress test was carried out and HTRIA genetic variation whose impact probably starts very early during development (although it might continue to influence serotonergic function over the course of life) resulted in considerable differences in stress regulation much later in life. Despite all inconsistencies, which remain to be resolved in follow-up studies, our data do not only point to a role of genetic variation in the modulation of the stress response but also add to the growing evidence on the impact of SLEs on stress regulation.

Conflict of interest

None of the authors has a conflict of interest to declare.

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