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Gene—environment interactions predict cortisol responses after acute stress: Implications for the etiology of depression

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Summary
Background: Growing evidence suggests that the serotonin transporter polymorphism (5-HTTLPR) interacts with adverse environmental influences to produce an increased risk for the development of depression while the underlying mechanisms of this association remain largely unexplored. As one potential intermediate phenotype, we investigated alterations of hypothalamic—pituitary—adrenal (HPA) axis responses to stress in individuals with no history of psychopathology depending on both 5-HTTLPR and stressful life events.
Methods: Healthy male adults ($N = 100$) were genotyped and completed a questionnaire on severe stressful life events (Life Events Checklist). To test for gene-by-environment interactions on endocrine stress reactivity, subjects were exposed to a standardized laboratory stress task (Public Speaking). Saliva cortisol levels were obtained at 6 time points prior to the stressor and during an extended recovery period.
Results: Subjects homozygous for the s-allele with a significant history of stressful life events exhibited markedly elevated cortisol secretions in response to the stressor compared to all other groups, indicating a significant gene-by-environment interaction on endocrine stress reactivity. No main effect of either 5-HTTLPR (biallelic and triallelic) or stressful life events on cortisol secretion patterns appeared.
Conclusion: This is the first study reporting that 5-HTTLPR and stressful life events interact to predict endocrine stress reactivity in a non-clinical sample. Our results underpin the potential moderating role of HPA-axis hyper-reactivity as a premorbid risk factor to increase the vulnerability for depression in subjects with low serotonin transporter efficiency and a history of severe life events.

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1. Introduction

Development of major depression is modulated by a complex interplay of both genetic and environmental influences (Lesch, 2004). Therefore, a major challenge of current research remains to identify specific risk factors. Since serotonin (5-Hydroxytryptamine, 5-HT) plays a crucial role in mood disorders and their treatment (Nemeroff, 2002), genetic association studies on depression have focused on allelic variations within genes coding for important regulatory components of serotonergic neurotransmission. Thereby, particular attention has been paid to a 44 bp insertion/deletion polymorphism (5-HTTLPR) within the serotonin transporter gene (SCL6A4), referred to as the long (l) and short (s) alleles (Lesch et al., 1996). Concerning the functional consequences, in vitro studies demonstrated a marked reduction of serotonin transporter (5-HTT) transcription efficiency and 5-HT uptake functioning in carriers of the s-allele (Greenberg et al., 1999; Lesch et al., 1996; Stoltenberg et al., 2002), even though conflicting results have been obtained especially with regard to in vivo studies (Heinz et al., 2000; Parsey et al., 2006; van Dyck et al., 2004) Observed inconsistencies could at least partly be due to unrecognized allelic variation, since the detection of a functional A/G polymorphism (rs25531) within the l-allele (Nakamura et al., 2000) suggests the 5-HTTLPR to be functional triallelic. More precisely, carriers of the L_G variant were characterized to exhibit a 5-HTT efficiency comparable to those carrying the s-allele (Hu et al., 2006; Praschak-Rieder et al., 2007; Reimold et al., 2006).

Numerous studies reported a significant association between the s-allele and major depression (Collier et al., 1996; meta-analysis: Lotrich and Pollock, 2004), although subsequent studies often failed to replicate these initial findings (Serretti et al., 2002; meta-analysis: Lasky-Su et al., 2005). Since environmental influences are known to relate to the incidence of depression as well (e.g. stressful life events: Heim et al., 2004; Kendler et al., 1999) it is promising to combine both aspects in candidate gene approaches. In fact, a prominent study by Caspi et al. (2003) demonstrated that only those carriers of the s-allele with a history of severe life events showed an increased risk for the development of depression, indicating a significant gene-by-environment (G × E) interaction. Despite the fact that this initial finding has been replicated with remarkable consistency (e.g. Kaufman et al., 2004; Kim et al., 2007; see Uher and McGuffin, 2008 for review), the mechanisms by which G × E interactions influence depression remain largely unexplored.

As suggested by Kendler et al. (2005), increased stress sensitivity to ''common, low-treat events'' in subjects with the s/s genotype and high life stress could be one potential moderating endophenotype. This hypothesis seems particularly interesting with regard to stress-induced hypothalamic–pituitary–adrenal (HPA) axis reactivity, since patients with major depression are characterized by multiple dysregulations of HPA-axis activity (see Holsboer, 2001; Plotsky et al., 1998 for review). In turn, alterations of endocrine stress reactivity depending on the 5-HTTLPR genotype can be assumed given the important role of serotonergic neurotransmission in activation and feedback control of HPA-axis (see Fuller, 1990; Porter et al., 2004 for review). Indeed, mice with targeted disruption of the 5-HTT gene are characterized by an exaggerated HPA-axis response to acute stress (Li et al., 1999). Similar results have been obtained in a recent study on children with a family history of depression, where the s/s genotype was associated with elevated cortisol responses (Gotlib et al., 2008). Given the fact that twin studies suggest only moderate heritability of HPA-axis reactivity to psychosocial stress (Federenko et al., 2004; Kirschbaum et al., 1992), the role of environmental factors needs to be considered as well. Stressful life events (SLEs), especially childhood maltreatment, have been associated with both hypo- and hyper-reactivity to acute stress (Carpenter et al., 2006; Elzinga et al., 2008; Heim et al., 2002; Rao et al., 2008). Therefore the question arises, whether the influence of life events on HPA-axis reactivity to acute stress is modulated by genetic make up, like 5-HTTLPR genotype. Although this G × E approach has proven to be fruitful in research on depression, association studies relating to potential risk factors for major depression – like an altered HPA-axis reactivity – have mainly been carried out in animals. For example, in rhesus monkeys the rh5-HTTLPR s-allele is associated with an exaggerated HPA-axis response to acute stress only when animals grew up under stressful rearing conditions (Barr et al., 2004).

In humans, additional evidence for increased stress sensitivity in s-allele carriers is provided by fMRI studies on emotion regulation. In these studies the s-allele is associated with increased amygdala reactivity to fearful stimuli compared to neutral ones (Hariri et al., 2002; meta-analysis: Munafò et al., 2007). However, some authors suggest that this differential activation pattern is mainly driven by a decreased amygdala activation to neutral stimuli relative to a fixation cross condition (Canli et al., 2005; Heinz et al., 2000). As potential explanations for this phenomenon increased amygdala activity at rest (Canli et al., 2005) as well as greater amygdala reactivity to an ambiguous and uncertain stimulus like the fixation cross (Heinz et al., 2007) have been suggested. Referring to the problem of defining an adequate baseline condition in fMRI studies, a recent report on rhesus monkeys demonstrated that rh5-HTTLPR s-allele carriers show elevated amygdala activity during the acute stress of relocation compared to a non-stressful homecage condition (Kalin et al., 2008). These results add support to the notion that increased amygdala reactivity in s-allele carriers is indeed due to a heightened response to stressful stimuli. Findings on amygdala reactivity to stressful stimuli were further extended by studies showing that s-allele carriers exhibit decreased gray matter volume of amygdala and anterior cingulate together with reduced functional coupling between those structures (Pezawas et al., 2005). To date, there is already one study demonstrating that the effects of 5-HTTLPR on amygdala activity are a function of SLEs as well (Canli et al., 2006). Elevated amygdala reactivity to aversive, stressful stimuli can in turn lead to an exaggerated stress response, since the amygdala plays a crucial role in stimulating HPA-axis activity (see Herman et al., 2005 for review).

Based on these previous findings, the aim of our study was to demonstrate that HPA-axis reactivity depends on both: The 5-HTTLPR genotype *and* stressful life events in a non-clinical sample.