The role of the serotonin transporter polymorphism for the endocrine stress response in newborns

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Summary A functional polymorphism in the 5’flanking region of the serotonin transporter gene (17q11.2, 5-HTTLPR) alters the transcription of the 5-HT transporter gene and seems to be associated with depression and anxiety-related personality traits in humans. This effect appears to be the most pronounced in individuals who are homozygous for the low-expressing “S” allele who have experienced significant critical life events in the past. Animal studies now link this polymorphism to an increased stress reactivity of the hypothalamus–pituitary–adrenal (HPA) axis. In humans, it remains unknown whether this polymorphism by itself affects HPA axis or only in interaction with environmental factors. The aim of the present study was to investigate the role of the 5-HTTLPR polymorphism for the HPA axis in humans early in the development at a time when individuals were exposed to very few or no early adverse experiences so far.

We genotyped DNA for the 5-HTTLPR polymorphism including the A/G single-nucleotide polymorphism (SNP) in 126 three-day old newborns. The newborn’s stress response was stimulated by a heel prick which is a part of a routine medical procedure. The heel prick induced a significant biological (i.e., cortisol) stress response in all newborns. Newborns with the “S/S” genotype showed a significantly higher endocrine response in comparison to newborns with “L/L” or “S/L” genotype.

In this sample of newborn babies, the 5-HTTLPR genotype affected the HPA stress response to painful stimulation irrespective of additional influence of pre- or perinatal environmental factors we measured.

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1. Introduction

An increasing number of studies links genetic variations with psychiatric disorders. Besides, the meaningful variation in DNA sequences for instance single-nucleotide polymorphism...
(SNP) may alter mRNA stability or the amino acid sequence which then can then result in a modified gene product. Allele variants in regulatory regions however, may alter transcription factor binding or act via further regulatory mechanisms which may result in a changed quantity of expressed gene products. These polymorphisms appear to have a significant impact on neurotransmitter systems in the brain which can have significant consequences for cognitive or emotional processing as well as psychopathology. Among the most frequently studied neurotransmitter systems with respect to emotional and behavioral endophenotype differences is the brain serotonin system. It is pivotally involved in emotional processes, and dysregulation of the serotonergic system has been described in several psychopathological disorders such as depression (review in Jans et al., 2007), social anxiety disorder (Matthew and Ho, 2006), posttraumatic stress disorder (Vieweg et al., 2006), or obsessive–compulsive disorder (Ninan, 2003).

A functional polymorphism (5-HTTLPR) in the 5′flanking region of the serotonin transporter gene (5-HTT, 17q11.2) alters gene transcription of the serotonin transporter and seems to be associated with depression as well as with anxiety-related personality traits in humans (Casp et al., 2003; Hariri et al., 2005; Lesch et al., 1996; Sen et al., 2004). Likewise, an association of this polymorphism with variations in the endocrine stress reactivity of the hypothalamus—pituitary—adrenal (HPA) axis was described in animals (Barr et al., 2004b) and in humans (Caspi et al., 2003; Kendler et al., 2005; Neumeister et al., 2002). Caspi et al. (2003) described that individuals with at least one S allele of the 5-HTTLPR and early adverse experience (e.g., childhood abuse) showed an increased risk for major depression or suicidal ideation. In contrast, individuals with “L/L” genotype were not at greater risk for depression irrespective of the number of experienced critical life events (Caspi et al., 2003). Additionally, Anor et al. (2004) revealed that genetic polymorphisms that reduce 5-HTT expression might impact on the early development of the central nervous system (CNS) which subsequently can modify emotional responses to stress (Anor et al., 2004). Thus, individuals with the “S” allele seem to be vulnerable to develop depression when exposed to a number of critical life events. While other groups replicated these findings (Benett et al., 2002; Eley et al., 2004; Kendler et al., 2005; Neumeister et al., 2002), conflicting results also exist (Gillespie et al., 2005; Ohara et al., 1998; Surteens et al., 2006).

The hypothalamus—pituitary—adrenal (HPA) axis might be an important mediator of the association between the 5-HTTLP and psychopathology as alterations of the HPA axis are linked to psychopathology, e.g., depression or anxiety disorders (Chrousos, 2000; McEwen, 2005; Selye, 1936). Although the causal relation between HPA axis alterations and psychopathology remains unclear, it might be hypothesized that individual differences in HPA axis activity influence the individual’s vulnerability for psychopathology (Dallman et al., 1987; Fries et al., 2005). The HPA axis is activated in response to a multitude of stimuli. Corticotropin-releasing hormone (CRH) is released from neurons in the paraventricular nucleus (PVN) of the hypothalamus stimulating the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary. Subsequently, ACTH reaches the adrenal glands stimulating the synthesis and release of species-specific glucocorticoids. Glucocorticoids influence metabolic and immune processes, adapting the organism to changing demands and promoting organism’s survival (Chrousos, 2000; Chrousos and Gold, 1992; Kirschbaum and Hellhammer, 1989).

As HPA axis activity is under partial control of the serotonergic system (Fuller, 1996; Lowry, 2002), this axis might be influenced by the 5-HTTLPR polymorphism. In a study with rhesus macaques, Barr et al. (2004b) showed an association between the “S” allele of the polymorphism and dysregulation of HPA axis functioning with early environmental conditions influencing this association. Rhesus macaques with at least one “S” allele of an analogous serotonin transporter polymorphism (rh5-HTTLPR) demonstrated an increased secretion of ACTH in response to stress when they experienced adverse rearing conditions during childhood. Conversely, rhesus macaques with at least one “S” allele and without critical rearing conditions showed a normal ACTH response to separation stress (Barr et al., 2004b). The study of Barr et al. (2004b) underscored the importance of environmental factors for the association between 5-HTTLPR and psychopathology. A relative reduction of 5-HTT gene transcription is linked with an increased risk for psychopathology in association with the early adverse (Casp et al., 2003; Hariri et al., 2006; Kendler et al., 2005; Neumeister et al., 2002). Caspi et al. (2003) described that individuals with at least one S allele of the 5-HTTLPR and early adverse experience (e.g., childhood abuse) showed an increased risk for major depression or suicidal ideation. In contrast, individuals with “L/L” genotype were not at greater risk for depression irrespective of the number of experienced critical life events (Caspi et al., 2003).
showing a gene—environment interaction (Alexander et al., 2009). This might be due to the fact that in both, animal and human studies the 5-HTTLPR genotype effects were studied with individuals being well into childhood, adolescence, or adulthood, respectively. To sum up, these contradictory gene—environment interaction results might have evolved from unreliable measures of environmental factors (Uher and McGuffin, 2008).

In the present study, we therefore investigated the main effect of the 5-HTTLPR genotype on the endocrine stress response in a sample with a history of no or very few early adverse experience. We hypothesized that healthy newborn babies show similar baseline cortisol levels and a comparable endocrine stress response to a stressful medical check-up (heel prick) irrespective of the 5-HTTLPR genotype. In addition, we exploratory tested whether 5-HTTLPR impacts on endocrine stress response in interaction with early adverse experience.

2. Materials and methods

2.1. Participants

Between May 2005 and August 2006, women staying in the Medical Clinic “Carl Gustav Carus” of the Technische Universität Dresden (Germany) were invited to participate in this study. Twenty-four hours after delivery of their child, mothers were informed about the study and asked for written informed consent in the hospital. In order to assess the newborns’ endocrine stress reactivity, saliva for cortisol determination was sampled before and after a routine neonatal screening test consisting of a heel prick performed by medical personnel three days after delivery. The heel prick is routinely conducted in order to obtain a capillary blood sample for metabolic disease check. Previous studies showed that the heel prick is a significant stressor inducing elevations of cortisol in newborns (Buske-Kirschbaum et al., 2004; Gunnar et al., 1988; Mantagos et al., 1991). The heel prick was performed in the morning around 0600 h.

A total of 244 newborns were initially included in the study. Insufficient saliva volumes for the first and/or second sample (N = 82), statistical outliers with a baseline ≥40 nmol/l (N = 32) as well as outliers with postheep prick cortisol values of ≥100 nmol/l (N = 4) were excluded and thus, reduced the final study sample to N = 126 three-day old newborns (72 females; 54 males) for endocrine data. Five preterm newborns were included in the sample as no stress response differences were observed between preterm and term born children. The study protocol was approved by the Ethics Committee of the Dresden Medical Clinic “Carl Gustav Carus” of the Technische Universität Dresden (Germany).

The 126 newborns had a mean gestational age of 40 weeks (SD = 1.27; range = 37–42 weeks). Five preterm newborns were born at gestational week 37 with no indication for special treatment as e.g., incubators after delivery. The mean birth weight was 3467 g (SD = 425.30; range = 2260–4500 g). 83 babies were born by vaginal delivery, 28 by caesarian section. In 15 cases information on mode of delivery was missing. The mothers’ mean age was 29 years (SD = 4.93; range = 18–40 years). No mother had preeclampsia or eclampsia, abused drugs or alcohol during pregnancy, 11 women continued smoking during pregnancy, 55 mothers reported medical conditions during pregnancy: 8 reported bleedings, 6 had infections, 4 had diabetes, 3 vomited excessively, 1 developed hypertension. 26 mothers reported sleeping difficulties and 6 women other conditions not further specified.

2.2. Psychological and sociodemographic assessment of the mothers

Mothers were asked to fill in the following questionnaires on the day of the heel prick: the Perceived Stress Scale (PSS) was applied to measure the perception of stress during the last month (Cohen and Williamson, 1988) and the Trier Inventory for the Assessment of Chronic Stress (TICS) assessed the subjective rating on experienced chronic stress during the last three months (Schulz et al., 2004). Furthermore, a general questionnaire about sociodemographic status, mode of delivery, nutrition and psychological condition including a global subjective rating on stress during pregnancy was given. Mothers were asked to appraise their subjective rating on stress during pregnancy, i.e., whether they experienced stress or not on a dichotomized scale (yes vs. no).

2.3. Collection of newborns’ saliva samples

Saliva samples were collected using medical applicators (Applimed SA, Châtel-Saint-Denis, Switzerland) 10 min before and 20 min after the heel prick. For this, the newborn’s mouth was gently swept with a medical applicator for approximately 30 s collecting saliva from the oral cavity. Samples were stored at −20 °C until analysis. Before biochemical analyses, samples were prepared by centrifuging at 3000 rpm for 3 min to obtain clear supernatant with low viscosity. Salivary free cortisol levels were determined employing a commercial chemiluminescence immunoassay (CLIA, IBL; Hamburg, Germany) with high sensitivity of 0.5 nmol/l. Intra- and interassay coefficients of variation were below 8%.

2.4. Genotyping

To obtain DNA samples, foam-tip buccal cell collection swabs (Epigenet, Madison, USA) were gently swept along the newborn’s oral mucosa. Genomic DNA was isolated using a quick extraction DNA 1.0 lysis buffer solution (Epigenet, Madison, USA). Genotypes for 5-HTTLPR including the A/G SNP were determined as described earlier (Lesch et al., 1996; Wendland et al., 2006).

Primer sequences previously described were applied (Lesch et al., 1996) and polymerase chain reaction (PCR) was carried out. Five thermocyclings were conducted and after a final extension genotypes were resolved. The serotonin transporter gene (SLC6A4; 5-HTT) was genotyped for the 44 sbp/del 5-HTT-linked polymorphic region (5-HTTLPR). The A/G SNP was detectable after digestion with an enzyme and electrophoresis on agarose gel containing ethidium bromide.

Comparable to other studies on European subjects (Strobel et al., 2007), 65.9% of the newborns had at least one “S” allele (including SS, LdLa, SLa, LdLa, and SLa) and 34.1% had both “L” alleles (“LdLa”). Model frequencies of the three 5-
HTTLPR genotypes including A/G SNP revealed that 43 newborns (34.1%) were homozygous for the "L" allele ("L/L"), 37 newborns (29.4%) were homozygous for "S" allele ("S/S"), and 46 (36.5%) newborns were heterozygous for "S" and "L" allele ("S/L"). Genotype distribution was in Hardy–Weinberg equilibrium for male newborns (p = .28), whereas it was not for female newborns (p < .05). The genotype distribution was therefore not in Hardy–Weinberg equilibrium for the total group of newborns (p < .05).

2.5. Statistical analyses

We conducted ANOVA for repeated measures with time point of saliva sampling (pre- and postheel prick) as within-subject factor and genotype ("S/S" vs. "S/L" vs. "L/L") as between-subject factor. For investigating possible group differences for gender or birth weight, one-way-ANOVAs were applied.

To analyze the possible impact of prenatal environmental factors on the endocrine stress response, ANOVAs for repeated measure were conducted with the cortisol data, respectively, as within-subject factor and genotype, mothers’ global subjective rating on stress during pregnancy and ratings on stress questionnaires as between-subject factors.

For the measure of experienced stress during pregnancy, the sample was divided into two groups with comparing mothers who experienced no stress at all during pregnancy with those who reported stress. The groups did not differ with regard to genotype (p = .51). For the measure of mothers’ chronic stress, the sample was divided into four groups regarding the sum scores of TICS and PSS questionnaires. These groups did not differ with regard to genotype (all p > .24).

Likewise, ANOVAs for repeated measures were conducted analyzing the possible impact of perinatal environmental factors as birth weight and mode of delivery on the endocrine stress response. Cortisol data were entered as within-subject factor and genotype, mode of delivery and birth weight as between-subject factors. For these analyses, with respect to weight the sample was divided into four groups. These groups did not differ with regard to genotype (p = .13).

Bonferroni adjusted post hoc analyses and follow-up ANOVAs for group comparisons were conducted where appropriate. For determination of effect size, partial Eta² for significant effects was estimated. Degrees of freedom were Greenhouse–Geisser corrected where appropriate. Level of significance was p < .05 for all analyses. All statistical analyses were performed using SPSS for Windows (Version 12, Chicago, IL, USA).

3. Results

Genotype groups did not differ regarding birth weight (F(6,121) = 3.67, p = .29), mode of delivery (χ²(2,126) = 2.84, p = .24) or gender (χ²(2,126) = 1.84, p = .50) as noted in Table 1. No differences between genotype groups regarding gestational week have been found either (F(2,119) = 2.20, p = .12). Moreover, the endocrine stress response was not influenced by gender (F(1,126) = 0.38, p = .57).

With a tendency of a significant difference in baseline cortisol levels (pre-heel prick) between 5-HTTLPR genotype groups (F(2,126) = 2.67, p = .07), the heel prick induced a profound increase of cortisol in all newborns (F(1,126) = 69.63, p < .001, η² = 0.36). Further repeated ANOVAs revealed a significant main effect for the 5-HTTLPR genotype on mean cortisol levels (F(2,126) = 4.01, p < .05, η² = 0.06) showing a higher stress response in newborns with "S/S" compared to newborns with the "L/L" genotype (p < .05; Fig. 1). A tendency for a significant difference was found between newborns with "S/S" and "S/L" genotype (p = .08), while there was no difference between "S/L" and "L/L" genotype (p = 1.00).

The endocrine stress response to the heel prick did not differ between male or female newborns (F(1,126) = 0.77, p = .38). Neither birth weight (F(1,121) = 0.95, p = .42) nor mode of delivery (F(1,111) = 1.37, p = .24) were associated with the newborns’ endocrine stress response.

Next, statistical ANOVA analyses were conducted investigating the possible influence of an interaction effect of genotype and prenatal environmental factors (maternal stress) on the endocrine stress response. Newborns’ endocrine stress response was not affected by a significant interaction between newborns’ genotype and prenatal environmental factors as assessed by mothers’ global subjective rating on stress (F(2,121) = 0.43, p = .65), or mothers’ subjective stress ratings as assessed with the PSS questionnaire (F(6,112) = 0.78, p = .59), or TICS (F(6,118) = 0.81, p = .57) as shown in Table 2.

Investigating the possible influence of perinatal factors on the association of the 5-HTTLPR and the endocrine stress response of the newborns, birth weight and mode of delivery were considered. No interaction between birth weight and newborns’ genotype influencing the newborns’ endocrine

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**Table 1** Information on gender distribution, birth weight (mean ± SD) and mode of delivery for the 5-HTTLPR genotype groups considering the single-nucleotide polymorphism (SNP) with an A to G substitution (dbSNP: rs25531) are displayed. One-way-ANOVAs and contingency tables were performed (providing F, χ² and p values).

<table>
<thead>
<tr>
<th>5-HTTLPR</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F or χ²</td>
</tr>
<tr>
<td>Females (n)</td>
<td></td>
</tr>
<tr>
<td>SS, SLG, LGLG</td>
<td>24</td>
</tr>
<tr>
<td>Males (n)</td>
<td></td>
</tr>
<tr>
<td>SS, SLG, LGLG</td>
<td>13</td>
</tr>
<tr>
<td>Birth weight (in g)</td>
<td>3345 ± 433</td>
</tr>
<tr>
<td>Type of birth (n)</td>
<td></td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>20</td>
</tr>
<tr>
<td>Caesarian section (n)</td>
<td>11</td>
</tr>
</tbody>
</table>
stress response ($F_{6,121} = 0.45, p = .84$) was observed. Furthermore, there was no significant interaction between mode of delivery and newborns’ genotype influencing the newborns’ endocrine stress response ($F_{2,111} = 0.36, p = .70$).

4. Discussion

This is the first study to examine the influence of a polymorphism in the serotonin transporter promoter region (5-HTTLPR) on the endocrine stress response to a routine medical examination in newborns. The pattern of findings presented here provides further evidence for the association of the serotonin transporter gene and HPA axis reactivity. Results indicate that the "S" allele in comparison to "L" allele increases biological stress reactivity stronger in humans, respectively.

Only few other studies have investigated the effect of 5-HTTLPR on the HPA axis in humans so far. Gotlib et al. (2008) examined a sample of 9–14-year old girls with a standardized laboratory stress task and showed stronger stress-related cortisol responses for girls with the "S" genotype than girls with at least one "L" allele, irrespective of risk for depression. In addition, Alexander et al. (2009) did not find a main effect of 5-HTTLPR on HPA axis functioning in a sample of male adults after a standardized laboratory stress task, whereas analyses including the history of adverse experiences revealed an elevated HPA-reactivity in newborns with the "S/S" genotype with a high number of adverse experience (Alexander et al., 2009). However, a number of other studies have confirmed the importance of gene—environmental interactions with regard to the vulnerability of psychopathological disorders (Barr et al., 2004a,b; Caspi et al., 2003; Kendler et al., 2005; Neumeister et al., 2002).

Recent work described the importance of adverse experience for the association of the 5-HTTLPR with anxiety, depression or an altered HPA axis reactivity (Barr et al., 2004a,b; Caspi et al., 2003; Kendler et al., 2005; Neumeister et al., 2002). In these studies, no main effect of the 5-HTTLPR polymorphism was observed. This is in contrast to the present findings in newborn babies. However, we assume that newborns carrying the "S" allele may be more vulnerable for, e.g., HPA axis disturbances and affective disorders after the experience of critical life events at a later stage of their lives.

The major difference between other studies and the present study is the relative lack of available detail regarding the infants’ prenatal are early postnatal environment. Although none of the babies here were known to have experienced early adversity (e.g., abuse, neglect), the impact of pre- or perinatal environmental factors (e.g., psychosocial stress or medical state of the mother (Welberg and Seckl, 2001)) on the newborns’ HPA axis functioning cannot be fully excluded. Chronic stress during pregnancy seems to have major impact on the fetus health as it has been shown to be associated with hyperactivity of the infants’ endocrine stress response (Maccari et al., 2003; Matthews, 2002; Weinstock, 2001). Increased ACTH and cortisol levels in stressed pregnant women are discussed to transmit the stress effects to the child (Wadhwa et al., 1996) as glucocorticoids, released from the maternal adrenal gland can partially pass the placenta and subsequently impact on the fetal organism (Gitau et al., 1998; Wadhwa et al., 2002; Weinstock, 2001). Thus, stress during pregnancy acts on the fetus and may communicate environmental influences from the mother to the fetus. Kraemer et al. (2008) recently studied this interaction in rhesus monkeys and showed an increased cortisol response to isolation from the mother only in offspring with

### Table 2

Association of the 5-HTTLPR genotype, mothers’ subjective stress ratings (number of mothers who experienced stress) and two questionnaires (including mean, SD and range) assessing chronic stress (PSS: Perceived Stress Scale and TICS: Trier Inventory for the Assessment of Chronic Stress) on the endocrine stress response in newborns. One-way-ANOVAs were performed (providing $F$ and $p$ values).

<table>
<thead>
<tr>
<th>Global stress rating</th>
<th>All subjects ($M \pm SD$ or $N$)</th>
<th>Association of 5-HTTLPR genotype and stress ratings on endocrine stress response ($F$)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress during pregnancy</td>
<td>27.0%</td>
<td>$F = .43$</td>
<td>.65</td>
</tr>
<tr>
<td>No stress during pregnancy</td>
<td>73.0%</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>PSS</td>
<td>19.64 ± 6.89</td>
<td>6–39</td>
<td>.78</td>
</tr>
<tr>
<td>TICS</td>
<td>14.28 ± 7.45</td>
<td>1–35</td>
<td>.81</td>
</tr>
</tbody>
</table>

Figure 1 Cortisol responses (mean ± standard error of mean) to heel prick stress, shown for the three genotype groups ("S/L" including $S/S$, $S/L_{LG}$, $L_{LG}/L_G$; "S/L" including $S/L_{LA}$, $L_{LA}/L_A$ and "L/L" including $L_{LA}/L_A$) in newborns.
at least one “S” allele and exposure to alcohol during pregnancy. In contrast to these results, no interaction between maternal stress and 5-HTTLPR on the endocrine stress response was observed in our sample of healthy newborns. It is possible that the prenatal stress exposure in our sample was too low to impact newborns’ HPA axis function.

Additionally, pregnancy-related experiences are affected by other behavioral, psychosocial, demographic, and environmental factors. The cortisol response to stress has also been associated with birth weight, that is a higher cortisol response to stress was observed in newborns with lower birth weight (Matthews, 2002; Seckl, 2004; Wust et al., 2005). In addition to prenatal factors, varying mode of delivery might depict an early adverse experience for the newborn (de Weerth and Buitelaar, 2007; Lu et al., 2008). Lower stress hormone concentrations and enhanced performed interactive processes have been found in infants after caesarean section in comparison to vaginally delivered newborns (Gathwala and Narayanan, 1990; Lu et al., 2008; Taylor et al., 2000). However, mode of delivery or birth weight was not associated with endocrine stress response in our sample.

There are some limitations to our study that need to be considered. First, the sample size was significantly reduced due to several relatively small because of outliers and insufficient saliva volume in a number of samples. Moreover, genotype distribution in our sample was not in Hardy–Weinberg equilibrium as we observed a shortage in the “S/L” genotype group. Even though others have detected Hardy–Weinberg equilibrium issues as well (Mandelli et al., 2007), our results need to be considered with caution. The shortage in the “S/L” genotype group would be one possible explanation for the marginal difference between “S/S” and “S/L” group instead of an expected significant difference between “S/L” and “L/L” group. Since we recruited an unselected sample of newborn babies, it is likely that the imbalance of genotype distribution has emerged by chance. In addition, our assessment of maternal stress only considered the last trimester of pregnancy, and assessment methods of perinatal stress factors were rather crude.

In summary, the results of the present study provide evidence that the HPA axis activity is significantly influenced by genetic variation of serotonergic function at a very early stage of life already. Further studies are needed to investigate the potential influence of prenatal and perinatal environmental factors for genotype effects in more detail, preferentially in a prospective study design.

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Conflicts of interest
There are no potential conflicts of interest or biomedical financial interests. There are no disclosures including direct or indirect financial or personal relationships, interests, and affiliations relevant to the subject matter of the manuscript that have occurred over the last two years. All authors have no conflicts of interest to declare.

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