Physiological reactivity to fear in children: effects of temperament, attachment & the serotonin transporter gene

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4 Electrodermal Reactivity during the Trier Social Stress Test for Children: Interaction between the Serotonin Transporter Polymorphism and Children's Attachment Representation

Abstract

This study explores the relation between variations in the serotonin transporter gene (5-HTTLPR; long vs. short allele), the child's attachment representation (assessed with the Attachment Story Completion Task, reflecting the security of the parent-child relationship), and electrodermal reactivity during a public speaking task, the Trier Social Stress Test for Children (TSST-C) in a sample of 92 7-year-old twins. Electrodermal reactivity during the TSST-C was not directly associated with variations in 5-HTT. However, there was a significant gene-environment interaction effect of 5-HTT and attachment security on electrodermal reactivity. Results are interpreted in terms of cumulative protection: Children with a secure attachment representation as well as long 5-HTT alleles appeared to be less stressed during the TSST-C.

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Introduction

Why do children differ in their reactivity to stressful settings such as a public speaking task or a difficult arithmetic test? The quality of the parent-child relationship may be relevant in supporting the child to cope with stress (Cassidy, 1988; Cassidy & Shaver, 1999), but genetic differences might also come into play (Rutter, 2006). In the current study we examine individual differences in neurophysiological stress reactivity between children with more or less secure attachment representations, in relation to a specific genetic polymorphism (5-HTTLPR) that in previous studies has been shown to be associated with feelings of stress and anxiety. Of special interest is the interplay between genetic lay-out and attachment security in explaining some part of the variance in children's coping with stress (Belsky, 2005; Fox, Hane, & Pine, 2007).

The neurotransmitter serotonin (5-HT) has been associated with negative emotions, such as anxiety or stress, in both human and animal research (e.g. Munafo, Clark, & Flint, 2005; Sen, Burmeister, & Ghosh, 2004). Serotonin transporter alleles in the promoter region of the serotonin transporter gene (5-HTTLPR) occur in 2 variants; short (s) and long (l). The short allele of the serotonin transporter gene is known to make fewer serotonin transporter proteins (Heils et al., 1996). These proteins function as serotonin reuptake transporters from synapse to neuron (Fox, Hane, & Pine, 2007). Fewer serotonin transporter proteins lead to less serotonin reuptake, and the short 5-HTT allele is thus less efficient compared with the long allele. The aim of the current study was to examine whether in young children variations in serotonin transporter alleles and the level of experienced stress during a psychosocial stress task are directly associated, or whether 5-HTT variations interact with child characteristics that are of environmental origin, in particular the attachment representation of the relationship with the parents.

Public speaking is a common social fear (Furmark et al., 1999). A meta-analysis on the effectiveness of various paradigms to trigger neurophysiological stress reactivity concluded that uncontrollable social stressors with a negative evaluatory component were most effective (Dickerson & Kemeny, 2004). The Trier Social Stress Test (Kirschbaum, Pirke, & Hellhammer, 1993) is a procedure that includes both social-evaluative threat (speaking in the presence of an audience) and lack of control (arithmetic tasks that are impossible to complete within the time constraints). The combination of the public speaking and impossible cognitive tasks has indeed been shown to be an effective psychosocial stressor (Buske-Kirschbaum et al., 1997),

which elicits stronger physiological responses than other types of stressors (Dickerson & Kemeny, 2004). The Trier Social Stress Test for Children (TSST-C) is an adapted child version of the TSST, developed and evaluated by Buske-Kirschbaum et al. (1997). Buske-Kirschbaum and colleagues found increased physiological responses (cortisol level, heart rate) during the TSST-C in 9- to 14-year-olds (Buske-Kirschbaum et al., 1997) and in 7- to 12-year-olds (Buske-Kirschbaum et al., 2003). Similar results have been found by Gordis, Granger, Susman and Trickett (2006), who showed that both cortisol and α-amylase increased in response to the TSST-C in 10- to 14-year-old children.

The serotonin transporter gene (5-HTT) has been related to various types of stressors. People carrying the short allele have been shown to score higher on depression scales (e.g. Mössner et al., 2001) and to show more often anxiety-related personality traits (e.g. Katsuragi et al., 1999; Lesch et al., 1996) than individuals homozygous for the long allele. Hariri and colleagues (2002) found a direct relation between variations in serotonin transporter alleles and the activity of the amygdala, the brain structure critical for learning and responding to emotions. People with one or two copies of the short 5-HTT allele showed greater amygdala responses toward faces expressing fear or anger than people carrying two long alleles (Hariri et al., 2002). Similarly, Furmark and colleagues (2004) related 5-HTT allelic variation to amygdala activity in social phobia patients during a public speech task. Patients with a short 5-HTT allele showed increased amygdala activation compared with patients with two long alleles when speaking in public (Furmark et al., 2004).

Findings on associations between 5-HTT and anxiety-related behavioral phenotypes have, however, not always been consistent. For example, Middeldorp and colleagues (2007) found no evidence for a linear relation between 5-HTT and anxiety, depression, or neuroticism. Furthermore, Umekage and colleagues (2003) found no direct link between 5-HTT and anxiety-related personality traits. Even when possible explanations of the discrepancies (e.g. sample size, age, gender) were taken into account, Willis-Owen and colleagues (2005) did not find a direct association between variations in serotonin transporter alleles and neuroticism or depression. Meta-analyses on linear relations between 5-HTT and anxiety related traits have been inconsistent as well. In a meta-analysis by Munafo and colleagues (Munafo, Clark, & Flint, 2005) an association was found between 5-HTT and harm avoidance, but not between 5-HTT and neuroticism, while a meta-analysis of Sen and colleagues (Sen, Burmeister, & Ghosh, 2004) observed the opposite. The underlying mechanisms thus

seem to be quite complex, and the effects of 5HTT on behavioral phenotypes may be dependent on environmental factors.

It has been argued that an important next step in molecular genetics is to observe interactions between the serotonin transporter gene and environmental influences, such as the caregiving environment (Bakermans-Kranenburg & Van IJzendoorn, 2007; Propper & Moore, 2006; Rutter, 2006). Parent-child relationships, in particular, have been demonstrated to interact with genetic variations. Suomi (2003) reported the interaction between variations in 5-HTT and parenting in rhesus monkeys. Rhesus monkeys carrying a short allele showed the expected deficits in their behavior (extreme aggression, excessive alcohol consumption) only when they were raised by peers and not when raised by competent mothers (Barr et al., 2003). Fox and colleagues (2005) described an interaction effect between 5-HTT and maternal reports of social support. Behavioral inhibition towards peers was observed in children and related to variations in 5-HTT and mothers' perception of social support. Children with one or two copies of the short 5-HTT allele and low maternal social support were most likely to show inhibited behavior towards peers. Caspi and colleagues (2003) reported that the association between variations in 5-HTT and depression was moderated by stressful life events. Adults who experienced stressful life events and who carried the short allele were more likely to be depressed than adults homozygous for the long allele. Stein, Schork, and Gelernter (2007) showed an interaction between 5-HTT and childhood emotional or physical maltreatment. Children homozygous for the short allele with high levels of maltreatment showed higher levels of anxiety sensitivity, an endophenotype for anxiety disorders. Furthermore, Kaufman and colleagues (2004) reported that maltreated children carrying two short alleles and experiencing low support scored almost twice as high on depression ratings than maltreated children carrying a long allele or carrying two short alleles with positive support.

The purpose of the present study was to explore the relation between variations in the serotonin transporter gene (5-HTTLPR; long vs. short allele), the quality of the mother-child attachment relationship, and stress reactivity during the TSST-C. Currently, one of the best validated attachment measures during middle childhood is the Attachment Story Completion Task (ASCT; Verschueren & Marcoen, 1994; based on Bretherton, Ridgeway, & Cassidy, 1990, and Cassidy, 1988). Children's attachment representations as derived from their stories showed convergent validity with observational measures of the attachment relationship (Bretherton et al., 1990; Cassidy, 1988) and parental sensitivity (Goodman, Aber, Berlin, & Brooks-Gunn,

1998), indicating that they reflect the quality of caregiving environment and not a gene-based temperamental or cognitive child characteristic. We expected to find (1) no direct association between variations in serotonin transporter alleles and electrodermal reactivity during the TSST-C, but (2) that stress during the TSST-C as assessed with electrodermal reactivity would be dependent on the interaction between the serotonin transporter gene and the child's attachment representation.

Method

Participants

Ninety-two same-sex twin pairs participated in this study. Twins were recruited with the help of the Netherlands Twin Register (Boomsma, Orlebeke, & Van Baal, 1992). The group consisted of 43 pairs of boys and 49 pairs of girls. The mean age of the children was 7.4 years (*SD* = 0.3). Mean age of the mothers was 38.8 years (SD = 3.4), and they had completed 14.4 years of education (*SD* = 3.1). The mean age of the fathers was 41.1 (*SD* = 4.7), and fathers had completed 15.4 years of education on average (*SD* = 3.3).

Part of the twins (42%) had participated in previous research at 12 months of age (Bokhorst, Bakermans-Kranenburg, Fearon, van IJzendoorn, Fonagy, & Schuengel, 2003). The additional group of children were not different from the children who had participated in previous research on gender, χ^2 (1, *N* = 92) = 1.37, *p* = .24, maternal age, *t*(87) = -1.68, *p* = .10, or paternal age, *t*(86) = -.81, *p* = .42). None of the children had serious medical problems. All participants were born in the Netherlands. Permission for the study was obtained from the Committee for Medical Ethics of Leiden University Medical Centre and the Ethics Committee of the Faculty of Social and Behavioral Sciences of Leiden University.

Procedure

Mothers came to the laboratory with both twin children at the same time. The session consisted of two parts, in two separate rooms; one in which the Trier Social Stress Test for Children was performed and one in which attachment representations were measured. Twin children were separated from each other and switched after finishing one part for participating in the second part. The oldest child of each twin pair (Child 1) began in the room where the TSST-C was performed, the youngest (Child 2) in the room in which attachment representations were measured. All procedures were videotaped.

Trier Social Stress Test for Children (TSST-C)

Children were asked to perform a modified version of the Trier Social Stress Test for Children (TSST-C), developed and evaluated by Buske-Kirschbaum et al. (1997). As a combined public speaking/cognitive task, the TSST-C has been shown to be an effective psychosocial stressor (Buske-Kirschbaum et al., 1997; Dickerson & Kemeny, 2004). After a 2-minute break in which children could read a picture book, a female 'teacher' came into the room. She explained that the child had to deliver a talk by finishing a story in front of the teacher and 'the people behind the camera'. The story-beginning was translated from the story used by Buske-Kirschbaum et al. (1997). The children were read the beginning of the story by the teacher. They were told they had two minutes to prepare their story and that the story should be exciting and better than the stories of other children. During the 2-minute preparation period, the teacher was sitting behind the table and remained silent. After the preparation period the teacher repeated the story-beginning and told the children to try to finish it in two minutes. When children finished the story within the first 1.5 minute, the teacher urged the children in a supportive manner to elaborate their story for a maximum of three times. After the 2-minute talk, the teacher asked the child to solve a construction puzzle (Wiggly-Block) that is too difficult to complete for 7-year-olds in a 4-minute period and added that "most children of their age could solve it". After 4 minutes, the teacher left the room with the puzzle and told the child to wait for the judgment of the 'people behind the camera'. The subsequent judgment was positive for all children, thereby ending the stressful situation. The children were told by the experimenter how well they had performed during all the tasks; they were told that the teacher was mistaken, and they were given a certificate for their good job.

Electrodermal activity

Electrodermal activity (SCL) was measured to assess the amount of stress during the TSST-C. Depending on the sympathetic activity, sweat in the eccrine sweat glands decreases or increases, thereby influencing the level of electrodermal activity (Boucsein, 1992; Dawson, Schell, & Filion, 2000).

To measure electrodermal activity, two small Ag/AgCl electrodes were placed on the volar surfaces of the index and middle finger of the child's right hand. Electrodes were applied with a small amount of Unibase paste (Fowles, Christie, Edelberg, Grings, Lykken, & Venables, 1981) and taped onto the fingers with Leukoplast. The apparatus used was an ambulatory system called the Ambulatory Monitoring System (AMS; version 36, Vrije Universiteit, Department of Psychophysiology, Amsterdam, NL; e.g., Christie & Friedman, 2004). The combination of an Event Marker button on

the AMS together with the recording of time allowed us to synchronize the physiological reactions to the episodes of the TSST-C. The AMS recorded the specific time points and the beginning and end of each episode were marked with labels.

Attachment Story Completion Task

The security of the children's attachment representations was measured with the Attachment Story Completion Task (ASCT; Verschueren & Marcoen, 1994; based on Bretherton et al., 1990, and Cassidy, 1988). Using a child doll and a mother doll, children were asked to complete five attachment-related stories and three control stories (see Verschueren, Marcoen, & Schoefs, 1996, for a complete description of the stories). Each attachment-related story was coded as "secure"; "insecure-avoidant"; "insecure-bizarre/ambivalent," or -if the child did not tell a clearly secure or insecure story- "secure/insecure". Stories classified as "secure" contained descriptions of positive feelings and harmonious interactions between the child and her/his mother, without any negative, unclear or bizarre subjects or issues. Stories which showed negative, hostile, or bizarre interactions with the mother figure were classified as "insecure-bizarre/ambivalent". Stories with minimal interaction between mother and child, avoiding the topic, or reluctance to complete the story were classified as "insecure-avoidant".

Five independent coders, who were unaware of the physiological and genetic data, rated the verbal transcripts of the children's stories. Coders were trained and reliable on a set of 40 stories coded by dr Karine Verschueren (Leuven University). Intraclass correlations for the five coders on 40 stories ranged between .90 and .95. To reduce the possibility of an incorrect classification, all stories were coded twice by different coders. In cases of disagreement, a third coder decided. As prescribed by the coding system guidelines (Verschueren & Marcoen, 1994), children received an overall classification of their attachment representation as "secure" or "insecure" on the basis of the classification of the five stories. Alpha reliability of the five stories was modest (α = .59; for comparable alpha values of the stories, see Verschueren & Marcoen, 1999).

To test if story fluency was associated with security, word count was done on the three control stories. 80% of the control stories were transcribed. Security was not related to fluency (Child 1: $t(72) = -0.52$, $p = .60$; Child 2: $t(70) = -0.88$, $p = .38$). Furthermore, the fluency of the oldest children (Child 1) of each twin pair did not differ from the fluency of the youngest children (Child 2; $t(70) = 1.73$, $p = .09$).

DNA Genotyping

DNA samples of the children were collected with buccal swabs. The 5-HTTLPR polymorphism in the promoter region of the *SLC6A4* gene was genotyped by PCR amplification followed by agarose gel electrophoresis. Primer sequences were adopted from Gelernter, Kranzler, and Cubells (1997), the forward primer being 5'-ATGCCAGCACCTAACCCCTAATGT-3' and the reverse primer being 5'-GGACCGCAAGGTGGGCGGGA-3'. These primers are expected to produce a short fragment of 375bp representing the 14 repeat allele, and a long fragment of 419bp representing the 16 repeat allele. The nomenclature of the alleles is as suggested by (Heils et al., 1996), with the 375bp allele designated "s" (short) and the 419bp allele designated "l" (long). PCR fragments containing the 5-HTTLPR polymorphism were obtained in a total reaction volume of 25 μL, containing 50 ng of genomic DNA, 0.3 mM dNTPs, 1.5 mM $MgCl^{2+}$, 10 pmol of each primer and 0.3U of BioThermAB polymerase (Genecraft, Munster, Germany). PCR conditions were the following: an initial denaturation step of 10 min at 94°C, 36 cycles of 30 sec at 94°C, 1 min at 68°C and 1 min at 72°C, followed by a final extension step of 15 min at 72°C. The amplification products were separated on a 2% agarose gel with 0,001 % ethidium bromide and visualized by ultraviolet transillumination.

Due to unsuccessful genotyping 5-HTTLPR polymorphism data were missing in five first-born children and six second-born children. Children were categorized in two groups, depending on the existence of a short (ss or sl) or two long 5-HTT alleles (ll; e.g. Fox et al., 2005). Having a short or two long 5-HTT alleles was not associated with security of the attachment representation (Child 1: $p = .24$; Child 2: $p = .80$)

Analyses

Analyses focused on the first-born children of each twin pair (Child 1). We opted for the first-born children, because they are known to have experienced fewer perinatal problems and, therefore, resemble more closely the normal population. In addition, in our procedure the oldest twin siblings always started in the room where the TSST-C was performed, in contrast to the youngest children who began with the assessment of the attachment representations and performed the TSST-C about one hour later. The physiological data of the first-born twins were thus less liable to influences from other assessments. The distributions of attachment security (χ^2 (1, $N = 92$) = 0.05, $p = .84$) and 5-HTT genotype (χ^2 (1, $N = 87$) = 0.00, $p = .96$) were not different for the sub-groups of first-born versus second-born children.

Electrodermal responses during the TSST-C were calculated by subtracting the means during the baseline in which children read a picture book from the means during the four stress evoking episodes (prepare talk, talk, puzzle, wait), leading to reactivity scores (e.g. Buske-Kirschbaum et al., 2003). Physiological responses to stress are characterized by an increase in electrodermal activity, which would lead to positive reactivity values.

Results

Table 1 presents descriptive data of the main variables, and correlations with SCL-reactivity during the four stress-evoking episodes of the TSST-C. Boys showed more electrodermal reactivity during the puzzle task than girls ($t(90) = 2.12$, $p < .05$). Apart from attachment security, no other variable was significantly correlated to stress reactivity. Children with a secure representation of their attachment relationship were less reactive to the stress of the TSST-C (Prepare talk: $t(90) = 2.39$, $p < .05$; Talk: $t(90) = 2.21$, $p < .05$; Puzzle: $t(90) = 2.16$, $p < .05$; Wait: $t(90) = 2.17$, $p < .05$).

Table 1

Descriptive data of parent and child variables, and correlations with SCL-reactivity during the four stress-evoking episodes of the TSST-C.

	M	*SD*	*Prepare talk*	*Talk*	*Puzzle*	*Wait*
Age of child	7.4	0.3	.15	-.07	.06	.05
Male children (%)	46.7		.03	.16	.22*	.14
Age of mother	38.8	3.4	.06	-.04	-.12	-.06
Education mother (yrs)	14.4	3.1	.12	.10	.12	.21
Secure children (%)	55.4		-.24*	-.23*	-.22*	-.22*
5-HTT ll (%)	24.1		-.12	-.01	-.02	-.04

* $p < .05$

To examine the relation between the TSST-C and 5-HTT (ss / sl vs. ll), a repeated measures analysis of variance was conducted with the four stress-evoking TSST-C episodes as the within-subjects variables, and the presence of at least one short versus two long alleles as the between-subjects factor. Means and standard deviations of SCL-reactivity grouped by short versus long 5-HTT alleles are presented in Table 2. Repeated measures analysis revealed a significant main effect of time ($F(1, 85) = 16.60$, $p < .001$), no significant time*5-HTT interaction ($F(1, 85) = 0.79$, $p = .38$) and no significant main effect of the between-subjects factor, i.e. short

versus long 5-HTT alleles ($F(1, 85) = 0.28$, $p = .60$). Thus, no direct relation was found between 5HTTLPR and stress reactivity during the TSST-C as measured by electrodermal reactivity.

Table 2

Means and (standard deviations) of SCL-reactivity during the different stress evoking episodes of the TSST-C, for both the oldest (Child 1) as well as the youngest twin children (Child 2), homozygous for the long 5-HTT allele (ll) compared with those carrying a short allele (s).

TSST-C	Child 1		Child 2	
	s ($N = 66$)	ll ($N = 21$)	s ($N = 65$)	ll ($N = 21$)
	M (SD)	*M (SD)*	*M (SD)*	*M (SD)*
Prepare talk	1.56 (1.73)	1.05 (2.27)	1.65 (1.75)	1.54 (1.93)
Talk	2.63 (2.00)	2.57 (2.01)	3.37 (2.08)	2.92 (2.23)
Puzzle	1.80 (1.92)	1.70 (2.04)	1.90 (2.14)	1.64 (2.33)
Wait	1.88 (2.09)	1.69 (2.15)	1.81 (2.67)	1.94 (2.21)

To examine whether the representation of the attachment relationship with the mother interacted with 5-HTT variations during the TSST-C, a repeated measure analysis of variance was conducted with the four stress evoking episodes of the TSST-C as the within-subjects variables, and short versus long 5-HTT alleles and the attachment representation (secure vs. insecure) as between-subjects factors. A significant main effect of attachment was found, $F(1, 83) = 8.36$, $p < .01$, $\eta^2 = .09$. Children with an insecure attachment representation showed higher SCL-reactivity during the stress evoking episodes than children with a secure representation, see Table 3. This main effect of attachment was, however, qualified by a significant three-way interaction between TSST-C episodes, short versus long 5HTTLPR, and attachment ($F(1, 83) = 4.85$, $p < .05$, $\eta^2 = .06$). Children with two long alleles *and* a secure representation of attachment showed the lowest levels of stress reactivity during the TSST-C, in particular during the first episode, 'prepare talk', see Figure 1. Children with two long 5-HTT alleles appeared to be less stressed but only when they had a secure attachment representation (cumulative protection, see below). In a repeated measure analysis with gender as a covariate, the TSST-C episode*5-HTT*attachment interaction was still significant, $F(1, 82) = 4.58$. $p < .05$, as was the main effect of attachment security, $F(1, 82) = 6.31$, $p = .01$. Gender was not a significant covariate, $F(1, 82) = 1.44$, $p = .23$.

Table 3

Means and (standard deviations) of SCL-reactivity during the different stress evoking episodes of the TSST-C per security.

TSST-C	Child 1		Child 2	
	Secure	Insecure	Secure	Insecure
	(*n* = 49)	(*n* = 38)	(*n* = 45)	(*n* = 41)
	M (SD)	*M (SD)*	*M (SD)*	*M (SD)*
Prepare talk	1.08 (1.94)	1.90 (1.70)	1.37 (1.81)	1.90 (1.74)
Talk	2.28 (1.94)	3.05 (2.00)	3.16 (2.20)	3.38 (2.03)
Puzzle	1.43 (1.76)	2.21 (2.09)	1.78 (1.93)	1.90 (2.45)
Wait	1.44 (2.05)	2.34 (2.07)	1.55 (2.16)	2.17 (2.91)

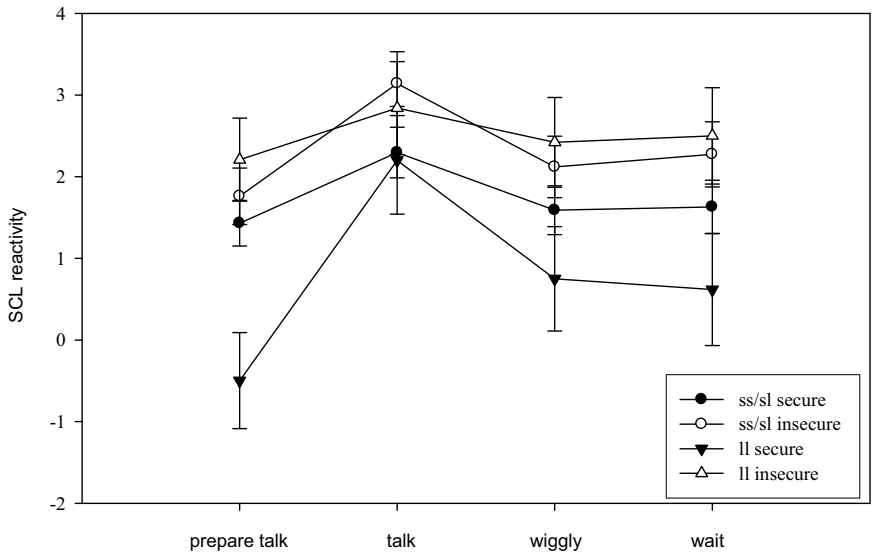

Figure 1. SCL-reactivity during the Trier Social Stress Test for Children with secure and insecure attachment representations, with and without the 5-HTT ll genotype

All analyses were repeated in the sample of second-born twin children, i.e. the youngest children of each twin pair. Again, repeated measures analysis of variance revealed a significant main effect of time ($F(1, 84) = 21.02$, $p < .01$), no significant episode*5-HTT interaction ($F(1, 84) = 1.37$, $p = .24$) and no significant main effect of

short versus long 5-HTT alleles ($F(1, 84) = 0.16$, $p = .69$). Thus, similar to the findings for Child 1, no direct relation was found between 5HTT and electrodermal reactivity during the TSST-C for Child 2. To replicate the finding of cumulative protection of two long 5-HTT alleles and attachment security, a repeated measure analysis was done with the four stress evoking episodes of the TSST-C for Child 2 as the within-subjects variables, and 5-HTT and attachment as between-subjects factors. For Child 2, the episode*5-HTT*attachment interaction pointed in the same direction of cumulative protection for children with two long 5-HTT alleles and a secure attachment representation, but the effect was non-significant, $F(1, 82) = 3.46$, $p = .07$, $\eta^2 = .04$. With gender as a covariate, the episode*5-HTT*attachment interaction was non-significant either, $F(1, 81) = 3.41$, $p = .07$.

Discussion

The present study demonstrates an interaction effect of 5-HTTLPR and attachment security on electrodermal reactivity during the Trier Social Stress Test for Children (TSST-C). Children with a secure attachment representation were less stressed than children with an insecure attachment. No direct association between variations in serotonin transporter genotype and electrodermal reactivity was found. However, the amount of stress during the TSST-C showed an interaction between variations in the serotonin transporter gene and attachment security. Children with two long 5-HTT alleles and a secure attachment representation showed the lowest levels of stress, in particular during the first episode of the TSST-C ('prepare talk'). These children thus appear to enjoy a dual protection; genetically (through a homozygous long 5-HTT genotype) and environmentally (through their secure attachment representation).

In line with our expectation, we did not find evidence for a direct relation between variations in serotonin transporter genotype and electrodermal reactivity during the TSST-C. This finding is consistent with previous results, showing no direct link between variations in 5-HTT and neuroticism, depression, or anxiety-related personality traits (e.g. Middeldorp et al., 2007; Umekage et al., 2003; Willis-Owen et al., 2005). However, the interplay between gene and environment (gene-environment interaction or GxE) that we found supports prior results indicating that variations in 5-HTT interact with different modes of parenting. In most previous studies, carrying a short allele of the 5-HTTLPR gene conferred a risk factor or vulnerability to psychopathology. Research by, for example, Caspi et al. (2003), Kaufman et al. (2004), and Stein et al. (2007) focused on depression or anxiety-related traits. Our study adds to these findings in that it focused on differences in stress reactivity

observed in a non-clinical sample, and the cumulative protection by carrying two long alleles and having a secure attachment representation is also a new finding. To our knowledge, this is the first study to explore the influence of a genetic factor in combination with (indirectly assessed) parenting on physiological reactivity during the TSST-C in typically developing children. The results indicate that in these children stress reactivity to a psychosocial stressor is buffered by the dual protection of both homozygous long 5-HTTLPR and a secure mother-child relationship.

Our study confirms that the TSST-C is an adequate and promising procedure to induce alterations in physiological activity in children. Previous studies with the TSST-C showed an increase in heart rate, cortisol levels (Buske-Kirschbaum et al., 2003), and α-amylase responses (Gordis et al., 2006) during the TSST-C in 7- to 14-year-old children. The increase in electrodermal activity that we found in 7-year-olds is comparable to these findings. The usefulness of electrodermal reactivity during stress- or fear-inducing tasks has been shown in other studies as well (e.g. El-Sheikh, 2007; Gilissen, Bakermans-Kranenburg, Van IJzendoorn, & Van der Veer, 2008).

Furthermore, this study supports the idea that attachment is a relevant factor in explaining individual differences. Attachment theory suggests that if a child is frightened, the activation of the fear system should be tempered by the (actual or represented) presence of a supportive attachment figure (Bowlby, 1973; Cassidy & Shaver, 1999). Securely attached children indeed showed less stress reactivity during the TSST-C than children with an insecure attachment representation, particularly when they carried the homozygous long variant of 5-HTT. It should be noted that children performed the TSST-C without their mothers, so even in the absence of the attachment figure physiological activation was less intense in securely attached children. Children with a secure representation or internal working model of attachment might be able to cope better with the stress evoking episodes of the TSST-C, because they have learned to feel safe and secure in similar stressful situations in the past. When parents have been sensitive and comforting in previous stressful situations, the child knows that once a situation becomes too stressful, the parent will be available for comfort if needed (Cassidy & Shaver, 1999). Also, research has documented the greater self-confidence of secure individuals who are less liable to be impressed by (undeserved) social disapproval (e.g. Cassidy, 1988; Mikulincer & Shaver, 2005; Verschueren, Marcoen, & Schoefs, 1996) that is important part of the TSST-C as well. It is one of the basic assumptions of attachment theory that trust in the attachment figure is fertile ground for the

development of trust in oneself as competent and able to cope with difficult situations (Cassidy & Shaver, 1999).

The current study is limited in various ways, and our findings should be replicated. First, the sample size for detecting reliable gene-environment interactions is moderate. The twin pairs offered a welcome opportunity to test and demonstrate the robustness of the findings in the second-born twin children but further independent replication is needed. Second, the quality of the children's attachment relationship with their mothers was measured with the Attachment Story Completion Task, which has been validated in various studies (Cassidy, 1988; Bretherton et al., 1990; Verschueren & Marcoen, 1994) but still leaves room for improvement in terms of the internal consistency of the children's responses to the five stories. Although Cassidy (1988) and Bretherton et al. (1990) showed that the attachment story completion task converged with concurrently applied observational measures of attachment, and Bretherton et al. (1990) documented the 'retrodictive' validity of this procedure against the Strange Situation and the Attachment Q-Sort, future studies should also include observations of parent-child interactions in order to tap more directly into the quality of the child's rearing environment. Third, the Trier Social Stress Test for Children is a powerful stressor, which fulfills established criteria for a stressor that affects neurophysiological functioning of the children involved (Dickerson & Kemeny 2004), and in our study we indeed found that the children showed elevated electrodermal responses to the TSST-C. Nevertheless, it would be important for future studies to examine the effects of various other, more naturally occurring stressors, in order to enhance the ecological validity of the findings. The advantage of using the TSST-C is the standardized nature of this stressor; for the sake of comparisons across studies it will turn out to be an indispensable tool for researchers studying stress reactivity in children.

Our focus in the current study was on 5-HTT polymorphism. The serotonin transporter gene is known to be a promising candidate gene involved in various anxiety-related personality traits (Lesch & Mössner, 1998). Carrying two long 5-HTT alleles is associated with increased serotonin reuptake compared to carrying one or two short 5-HTT alleles (Lesch & Mössner, 1998). The difference between the long and short 5-HTT alleles in serotonin expression and –reuptake appears to have its (direct or indirect) consequences on depression (Caspi et al., 2003), aggression (Suomi, 2003) and anxiety (Lesch et al, 1996). Our study supports the indirect relation between variations in serotonin transporter alleles and stress reactivity during

the TSST-C, and contributes to the growing literature on gene-environment interactions involving 5-HTT and quality of parenting.

It may be presumed, however, that other genetic polymorphisms play a role as well. 5-HTT is a reasonable candidate for modulating a stress response during the TSST-C, but variations in the GABRA6 gene polymorphism have also been shown to be associated with physiological responses to the Trier Social Stress Test (Uhart, McCaul, Oswald, Choi, & Wand, 2004). Individuals carrying the T allele showed greater physiological responses (cortisol, blood pressure, adrenocorticotropin) to the TSST than individuals carrying two C alleles. Furthermore, the dopamine D4 receptor (DRD4) has been shown to be an important polymorphism interacting with parenting quality. Bakermans-Kranenburg and Van IJzendoorn (2006) found that children with a DRD4 7-repeat allele (associated with lower dopamine reception efficiency) were more susceptible to sensitive or insensitive parenting than children without the DRD4 7-repeat allele. Children with the 7-repeat allele raised by highly sensitive mothers showed the lowest levels of externalizing behavior whereas the same children showed most externalizing behavior when raised by less sensitive mothers. In another study, Van IJzendoorn and Bakermans-Kranenburg (2006) showed that the risk for attachment disorganization was higher for children with the DRD4 7-repeat allele exposed to maternal unresolved loss or trauma than for children without the 7-repeat allele, and lowest for children with this allele but raised by mothers without unresolved loss or trauma (Bakermans-Kranenburg & Van IJzendoorn, 2007).

Interactive effects between genes are also plausible. Ebstein (2006) reviewed studies in which genetic polymorphism interactions (i.e. DRD4 x 5-HTT x COMT) contribute to various behavioral phenotypes (e.g. novelty seeking). Both the way in which genetic expression is affected by environmental influences as well as the complex processes that underlie genetic expression should be central in future studies (Rutter, 2006; Rutter, Moffit, & Caspi, 2006). Our results indicate a significant interaction effect of 5-HTT and attachment security on electrodermal reactivity in a stress evoking situation. We found evidence that in a non-clinical sample stress reactivity to a psychosocial stressor is characterized by the dual protection of both the more efficient homozygous long 5-HTT genotype and attachment security.

Gene-environment interactions are an important target for future research on child development. Behavioral geneticists have documented the rather large role of genetics in explaining behavioral differences in children (e.g. Plomin, Owen, & Mc Guffin, 1994), but it would be a mistake to de-emphasize the critical role of the

environment, and more particularly of the parents, in shaping their children's development (Rutter, 2006). Paraphrasing Urie Bronfenbrenner (1979), one might say that in child development main effects may have to be found in gene-environment interactions.