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

This thesis addressed the physiological impact of normal fears in 4- and 7-year-old children, induced by media and social fear-inducing tasks, and examined the individual differences in fear reactivity. The possibly relevant factors of attachment security, the child's temperamental fearfulness, and variations in the serotonin transporter gene were taken into account. Specific aims addressed in the thesis were, first, to determine the physiological effects of fear-inducing film clips in 4- and 7-year-olds; second, to examine whether the impact of fear-inducing film clips can be predicted by the quality of the parent-child relationship and/or the child's temperamental fearfulness; and third, to explore the relation between variations in the serotonin transporter gene (5-HTTLPR; long vs. short allele), attachment security, and the impact of a social fear-inducing task, the Trier Social Stress Test for Children.

Physiological impact of media-induced fear in 4- and 7-year-olds

Not many studies have been conducted on the physiological effects of media-induced fear in young children. The current thesis investigated physiological reactions to film clips in 4-year-olds (Chapter 2) and compared the impact of media-induced fear on 4-year-olds with its impact on 7-year-olds (Chapter 3). The two age groups were shown the same baseline, fear-inducing and neutral film clips. Children's electrodermal activity (to assess the sympathetic influence of the autonomic nervous system) and heart rate variability (to assess the parasympathetic influence) were recorded simultaneously. Reactivity scores were calculated by subtracting the means during the baseline film clip from the means during the fear-inducing or neutral film clips. Following our prediction, both 4- and 7-year-olds responded to the fear-inducing film clip with significantly more increases in electrodermal activity (sympathetic activation) and more decreases in heart rate variability (parasympathetic withdrawal) compared with their responses to the neutral film clip (Figure 1). The increases in electrodermal activity and decreases in heart rate variability during the fear-inducing film clip that we found correspond with the notion that physiological responses to fear are typically characterized by an increase in sympathetic- and a decrease in parasympathetic activity (e.g. Berntson et al., 1991). Furthermore, our results demonstrate the usefulness of assessing physiological reactivity during media-fear inducing tasks in young children.

The impact of media-induced fear, assessed with electrodermal activity, on 4- and 7-year-olds was comparable. However, heart rate variability reactivity differed between the two age groups. The 7-year-old children showed higher scores on heart rate variability in reaction to the fear-inducing and neutral film-clip, indicating less parasympathetic withdrawal than 4-year-olds. Perhaps older children were less stressed during the fear-inducing film clip (only expressed in parasympathetic activity). Another possibility might be that during the baseline film clip, 7-year-olds experienced more fear than during the neutral film clip, since the positive reactivity values during the neutral film clip represent higher heart rate variability during this film clip than during baseline. Baseline clips were shown just after children were told that they would see different kind of film clips. Maybe the expectations of the coming film clips were more arousing for 7-year-olds than for 4-year-olds, because older children are better able to imagine the possibly exciting fragments. Another reason for the positive reactivity values might be agitation of the older children during the baseline film clip. The baseline film clip showed stars and slowly turning mandalas in different colors. Perhaps 7-year-olds became more agitated during this film clip than 4-year-olds. The neutral film clip showed colorful objects (balls and ducks) moving in a slow rhythm and might have been a bit more entertaining.

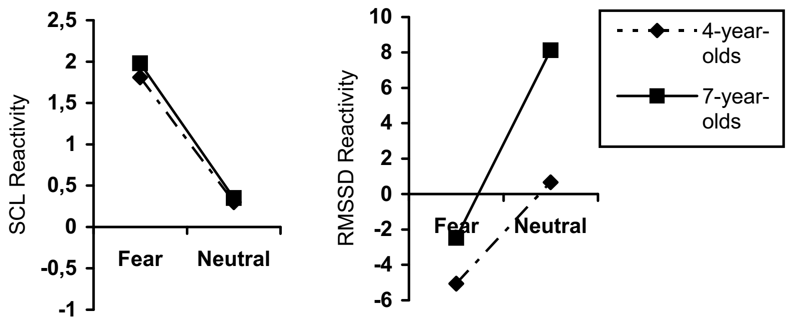

Figure 1. Ectrodermal- (left figure) and heart rate variability-reactivity (right figure) of 4- and 7-year-old children in response to a fear-inducing and neutral film clip

To be sure that the movie 'Dinosaur' was indeed fear evoking for the children, we coded particular behavioral signs of fear during watching (stimuli-blocking behaviors, such as covering the eyes) typically seen during high fear. About one third of the children showed these fear behaviors, implying that this film clip was indeed fearful for the children.

Media-induced fear: Effects of attachment security and temperamental fearfulness

Two potentially important causes of individual differences in physiological reactivity to fear-inducing film clips concern the relationship that a child has developed with its attachment figure and temperamental fearfulness. The possible prediction of physiological reactivity from these two factors as well as their interplay was studied. In line with our prediction, greater attachment security predicted less electrodermal reactivity to fear-inducing film clips. Of special interest, however, is the interaction that we found between attachment security and temperamental fearfulness. Temperamentally more fearful children appeared to be more susceptible to attachment security in their reactivity to fear-inducing film clips than less fearful children. This finding provides additional evidence for Belsky's differential susceptibility hypothesis (Belsky, 1997, 2005), which states that children with an emotionally "reactive" temperament are more susceptible to effects of parental rearing, with more negative as well as more positive outcomes, than emotionally robust children (Belsky, 1997, 2005). Indeed, more temperamentally fearful children with less secure relationships showed the highest electrodermal reactivities to the fear-inducing film clip, whereas comparable temperamentally fearful children with more secure relationships showed the lowest electrodermal reactivities (Figure 2). Our findings add to the growing literature showing that children high in negative emotion are more susceptible to positive as well as negative rearing influences, for better and for worse. The interaction of attachment security and temperamental fearfulness did not differ between 4- and 7-year-olds and was thus independent of children's age.

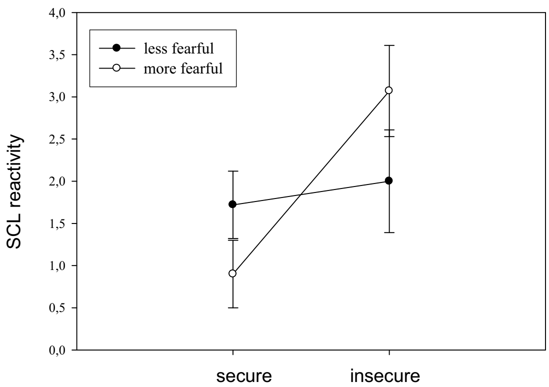

Figure 2. The relation between attachment security and electrodermal (skin conductance level; SCL) reactivity to fear-inducing stimuli for temperamentally less fearful and more fearful children

Unexpectedly, however, none of the predictors, nor the interaction, contributed significantly to the prediction of heart rate variability. Apparently, the interaction between temperamental fearfulness and attachment predicts only sympathetic reactivity. The difference between sympathetic and parasympathetic activity, which reflect different processes of the autonomic nervous system and have to be regarded as different and specific subsystems (e.g., Fox & Card, 1999), may account for the lack of prediction of (parasympathetic) heart rate variability. The correlation between electrodermal reactivity and heart rate variability reactivity proved to be weak (r = -.10, p = .22). Furthermore, the measure used to index heart rate variability (RMSSD) may be less precise compared to other measures assessing the parasympathetic influences on heart rate, like respiratory sinus arrhythmia (RSA; Berntson, 1997). RSA may be a more promising index of parasympathetic activity and may be used in future research on fear-inducing stimuli.

Social-induced fear: Effects of attachment security and 5-HTTLPR

Apart from media-induced fear, this thesis examined individual differences in fear reactivity in 7-year-old children during a social fear-inducing task; the Trier Social Stress Test for Children (TSST-C; Buske-Kirschbaum et al., 1997; Chapter 4). This procedure included four stress evoking episodes (preparing a talk: “Prepare talk”; performing the talk: “Talk”; solving a construction puzzle that is too difficult for 7-year-olds: “Wiggly”; and waiting for the judgment of ‘the people behind the camera’: “Wait”). Electrodermal activity was recorded during these episodes and reactivity scores were calculated by subtracting a baseline period in which children read a picture book. The possibly relevant factors of children’s attachment representation (secure vs. insecure) and variations in the serotonin transporter gene (5-HTTLPR; homozygous long vs. hetero- or homozygous short genotype) were examined. Children with an insecure attachment representation showed higher electrodermal reactivity during the TSST-C than children with a secure representation. No direct relation was found between variations in 5-HTTLPR and electrodermal reactivity during the TSST-C. However, our results indicated a significant interaction effect of variations in the serotonin transporter gene and attachment security. Children with a secure attachment representation and a homozygous long 5-HTT genotype showed the lowest levels of electrodermal reactivity during the TSST-C, particularly during the preparation of the talk (Figure 3).

In line with our expectation, we did not find evidence for a direct association between variations in the serotonin transporter genotype and electrodermal reactivity. The effects of 5-HTT appeared to be dependent on the children’s attachment representation. The interplay between a genetic factor and (indirectly assessed) parenting that we found supports prior results indicating that variations in 5-HTT interact with environmental factors (e.g. Caspi et al., 2003; Fox et al., 2005; Kaufman et al., 2004; Stein et al., 2007; Suomi, 2003). Our study adds to the previous findings in that it focused on differences in stress reactivity observed in a non-clinical sample. The results indicate that in these children stress reactivity to a psychosocial stressor is buffered by the dual protection of both the more efficient homozygous long serotonin transporter genotype and a secure attachment relationship.

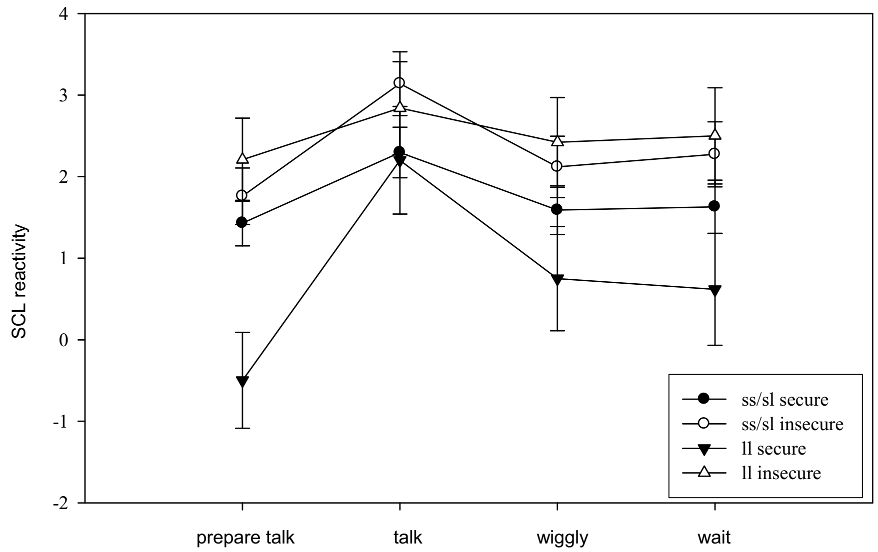

Figure 3. Electrodermal reactivity during the Trier Social Stress Test for secure and insecure children with and without the 5-HTT ll genotype

The results and their implications

The results of our studies should be interpreted and generalized with some caution. One debatable issue concerns the different measures used to measure attachment security. While the studies in this thesis support the idea that attachment is a relevant factor in explaining individual differences (securely attached children showed significantly less electrodermal reactivity than insecure children during both the fear-inducing film clips and the TSST-C), it might be argued that the Attachment Story Completion Task (ASCT; Verschueren & Marcoen, 1994) measures somewhat different aspects than the Emotional Availability Scales (EAS; Biringen et al., 1998). Beyond infancy, no"gold standard" measures of attachment security have been developed and validated (Solomon & George, 1999). Assessments of attachment security in preschoolers have been noted to present some difficulties. The attachment system is not easily activated, the behavioral repertoire is broader, and the verbal capacities are still limited and vary greatly between children and across situations (Solomon & George, 1999). One of the most promising and validated assessments to measure the quality of the parent-child relationship in preschoolers is the 'Emotional Availability Scales' (EAS; Biringen, et al, 1998). These scales have proven to be related to infant attachment security assessed with Ainsworth's (1978) Strange Situation Procedure (Easterbrooks, Biesecker, & Lyons-Ruth, 2000; Ziv, Aviezer, Gini, Sagi & Koren-Karie, 2000), and maternal Adult Attachment Interview classifications (Biringen et al., 2000).

In middle childhood, verbal capacities have improved and representational measures of attachment security for this age category have been developed (Solomon & George, 1999). Story stem measurements, like the Attachment Story Completion Task (ASCT; Verschueren & Marcoen, 1994; based on Bretherton et al., 1990, and Cassidy, 1988), are currently among the best validated attachment measures for middle childhood and have been shown to be associated with infant attachment security assessed with the Strange Situation Procedure (Bretherton et al., 1990), the separation-reunion procedure for preschoolers (Oppenheim, 1997), the Attachment Q-sort (Vaughn & Waters, 1990; Bretherton et al., 1990), and parental sensitivity (Goodman et al., 1998). The ASCT, as well as the EAS, have thus proven to be associated with more thoroughly validated measures of the parent-infant attachment relationship and of maternal attachment representations. Better attachment measures are currently not available.

In spite of this limitation, our study supports the idea that the serotonin transporter gene (5-HTTLPR) is a promising candidate gene involved in anxiety related personality traits (Lesch & Mössner, 1998; Lesch, 2003). The interplay between variations in 5-HTTLPR and attachment security that we found contributes to previous findings indicating that variations in 5-HTT interact with different modes of parenting. Children with a homozygous long 5-HTT genotype and a secure attachment representation showed the lowest levels of electrodermal reactivity during the TSST-C and thus seem to be best protected against the social fear stimulus. The difference between the long and short 5-HTT alleles in serotonin expression and – reuptake has been shown in prior studies to have its (direct or indirect) consequences on depression (Caspi et al., 2003; Kaufman et al., 2004), 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.

Furthermore, our studies confirm that fear-inducing film stimuli and the Trier Social Stress Test for Children are adequate and promising procedures to induce alterations in physiological activity in children. Standard procedures eliciting fear or distress in children are quite sparse. The small number of studies eliciting distress in young children may be the result of ethical considerations. One might question the use of fear-evoking stimuli in (young) children. The studies described in this thesis were approved by 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. Parents of the children were informed about the procedures beforehand and could withdraw their participation without explanation during all phases of the study. Furthermore, the media and social fear-inducing tasks used in our studies indeed do elicit distress but it is unlikely that they elicit more stress than children experience in everyday life. After all, the fear-inducing film clips included fragments of the children's movie "Dinosaur" (Marsden, 2000), an animation production of *Walt Disney*. This movie is categorized as suitable for all ages by "Kijkwijzer/Nicam" (Valkenburg et al., 2002), a Dutch classification system that gives age recommendations for television programs, movies, and video games. Kijkwijzer/Nicam warns of possible harmful images like violence, fear, sex, discrimination, and coarse language. The age recommendation "All ages" means *not harmful*. Our second stressor, assessed in 7-year-olds, included a public speech, which is a familiar situation to most school-aged children as well, because they know it from school. In most Dutch elementary schools, children are regularly required to deliver a speech about a self-chosen subject, even in the second and third grades.

While only few studies used stressors in research with children, in some research areas stress- or fear-inducing stimuli may actually be *necessary* to activate the behavior of interest. This seems especially true for studies on emotion regulation or attachment. Originally, measures to assess attachment were designed to activate the attachment system. In Ainsworth's Strange Situation Procedure (Ainsworth et al., 1978), 12- to 20-month-old infants are observed during two brief separations from and reunions with the caregiver. The separations are stress-evoking for the infants and typically activate the attachment system, which leads to attachment behaviors, like crying when the caregiver leaves the room and seeking for reassurance and physical contact when the caregiver returns. When children get older (preschool, middle childhood), assessments of attachment are primarily measured in other contexts that are often less stress-evoking. A separation from the caregiver may not be sufficiently arousing for these children (Solomon & George, 1999). Situations, in which less stress is elicited, however, have proven to be less adequate in activating the attachment system (Solomon & George, 1999). The degree to which these measures activate the attachment system is therefore questionable. For research on attachment in the preschool- and middle childhood years a search for effective stressors activating the attachment system and eliciting attachment behaviors that are essential for distinguishing the various attachment classifications may thus be important.

In studies on emotion regulation effective stressors are needed as well. Emotion regulation refers to emotional or psychological changes associated with activated emotions (Cole, Martin, & Dennis, 2004). Both physiological reactivity to a stressor and recovery from the stressor have shown to be important in understanding the process of emotion regulation (Hessler & Katz, 2007). An example of a study on emotion regulation that used stressors in laboratory assessments of children is that by Hessler and Katz (2007) in which physiological responses to peer provocation in 8- to 10-year-olds were studied. Children were asked to play a computer game against another child (an actor). They were told that it was a contest and that the winner of the contest would win a prize. However, the participant's keystrokes on the computer were artificially delayed and the child-actor made teasing comments after each game, like ”Why do you keep losing?” Children showed an increase in heart rate reactivity in response to the teasing comments, particularly when children scored higher on an emotion dysregulation scale (Hessler & Katz, 2007).

The demonstration of the usefulness of assessing physiological reactivity during media-and social-fear inducing tasks in young children is an important complement to previous studies. Most findings of media studies have emerged from observational studies or research based on questionnaires. However, observations may show discrepancies with physiological data. For example, children with different types of attachment insecurity might show different behavioral expressions independent of their physiological stress; avoidantly attached children might not show any behavioral expressions of their fear, because they have experienced that their parents reject them when they displayed negative emotions, and they do not take the risk of being rejected again. On the other hand, children who are ambivalently attached might show extreme fear expressions, hoping for a sensitive reaction of the inconsistently sensitive parent (Ainsworth et al., 1978). In future studies, the simultaneous measurement of facial expression and physiological reactivity (along with the various attachment relationships) might thus be preferred.

Fear is a common occurrence in daily life and it would not be reasonable to keep children away from all fearful situations, because they can learn from their experiences and gain knowledge of how to cope with fearful situations in the future. It is of great practical interest, however, to examine the possibly relevant factors that influence the impact on children. Children clearly differ in their stress reactivity to media clips and other potentially fearful stimuli or settings, and it is important to know what the characteristics are of the most vulnerable children. These children might need some extra buffering against detrimental effects of fear exposure, or they might be exposed to lower doses of fear stimuli, for example through more carefully monitored media consumption.

In sum, temperament, attachment, and genetic influences have been shown to play significant and interactive roles in the expression of fear reactivity. More temperamentally fearful children with less secure attachment relationships showed the highest fear reactivities to media-induced fear stimuli, whereas comparable temperamentally fearful children with more secure relationships showed the lowest fear reactivities. Furthermore, we found evidence that reactivity to a social fear-inducing task was explained by a combination of variations in the serotonin transporter gene and attachment security. Children with a secure attachment representation and two long 5-HTT alleles showed the lowest levels of fear reactivity, indicating that physiological reactivity to a social fear-inducing task is a product of the child’s underlying biology and environment