Infant attachment and stress regulation : a neurobiological study

Luijk, P.C.M.

2 Attachment, depression, and cortisol: Deviant patterns in insecure-resistant and disorganized infants

Maartje P. C. M. Luijk, Nathalie Saridjan, Anne Tharner, Marinus H. van IJzendoorn, Marian J. Bakermans-Kranenburg, Vincent W.V. Jaddoe, Albert Hofman, Frank C. Verhulst, and Henning Tiemeier (2010). Developmental Psychobiology, 52, 441-452.

Abstract

Both attachment insecurity and maternal depression are thought to affect infants' emotional and physiological regulation. In the current study, Strange Situation Procedure (SSP) attachment classifications, and cortisol stress reactivity and diurnal rhythm were assessed at 14 months in a prospective cohort study of 369 mother-infant dyads. Maternal lifetime depression was diagnosed prenatally using the Composite International Diagnostic Interview (CIDI). Insecure-resistant infants showed the largest increase in cortisol levels from pre to post SSP; the effect was even stronger when they had depressive mothers. Disorganized children showed a more flattened diurnal cortisol pattern compared to non-disorganized children. Findings are discussed from the perspective of a cumulative risk model.

Introduction

The infant-parent attachment relationship can be considered the infant's most important emotion regulation system (Bowlby, 1969/1982; Cassidy, 1994), since regulation is primarily externally organized in the first year of life. Early experiences are thought to shape the attachment relationship and thereby influence the regulation of behavioral and physiological responses. Most studies of the physiology of attachment relationships focused on measures of heart rate and cortisol during the Strange Situation Procedure (SSP, Ainsworth, Blehar, Waters, & Wall, 1978; e.g. Gunnar, Mangelsdorf, Larson & Hertsgaard, 1989; Oosterman & Schuengel, 2007; Sroufe & Waters, 1977). The current study includes the largest sample to date, which makes it possible to address issues of stress reactivity on the level of the various insecure attachment classifications. Furthermore, we examine the moderating role of maternal depression.

Early experiences have been shown to influence the behavioral and physiological organization of infants. Studies in humans and other animals

document that deprivation of care has a major impact on the infant's developing system of stress regulation (Boyce, Champoux, Suomi, & Gunnar, 1995; Caldji et al., 1998; Carlson & Earls, 1997; Levine & Wiener, 1988; Liu et al, 1997; Meaney, 2001; Plotsky & Meany, 1993). In relatively low-risk populations, differences in quality of care can predict differences in infant stress regulation. In the first year of life, regulation and coping are primarily externally organized. This makes the caregiver's responses to the infant's distress an important source of coping (Van Bakel & Riksen-Walraven, 2004). The availability of responsive, sensitive care is thought to promote infant attachment security and to mediate the infant's response to stressors (Gunnar & Donzella, 2002). Through their history of care, infants learn to what extent the caregiver is emotionally available in times of stress. Variation in parental availability, i.e. consistent sensitivity, inconsistent sensitivity, and consistent insensitivity, may lead to different secure and insecure attachment strategies in the infant (Sroufe, 1997).

Infants of consistently sensitive parents learn to expect their parents to be available in times of stress and have increased chances for developing a secure attachment relationship with their parent, which provides them with a powerful coping mechanism to regulate stressful stimuli. In contrast, infants of inconsistently sensitive or consistently insensitive parents do not come to expect their parents to be available in stressful situations. As a consequence, these children develop insecure attachment relationships with their parents. Insecure-resistant infants maximize their distress signals in order to get their parent's attention, whereas insecure-avoidant infants minimize signs of distress as they have learnt that they might be rejected (Main, 1990). In both cases the insecure children manage to create the best possible proximity to an attachment figure who is not optimally available. When the parent is extremely insensitive or even frightening, parental behaviors may cause a temporary breakdown in the child's strategy to keep close to the attachment figure which leads to dysregulation of negative emotions, as apparent in a disorganized attachment relationship (Main & Solomon, 1990).

Hertsgaard, Gunnar, Erickson, and Nachmias (1995) suggested that assessment of cortisol levels may be particularly useful in attachment research because the neuroendocrine system is believed to be stimulated when coping behaviors are inadequate or coping sources are unavailable. Studies on attachment quality and cortisol have focused mainly on stress reactivity, with assessment of cortisol levels before and after a potentially stressful event. The Strange Situation Procedure has often been used as the stressful event, as it is based on a series of brief infant-caregiver separations and reunions. The SSP is the gold standard procedure to assess the quality of the attachment relationship. Other methods of observing attachment quality, such as the Attachment Q-set (AQS; Waters, 1995) have not been widely used in cortisol research (but see Oosterman & Schuengel, 2007; Van Bakel & Riksen-Walraven, 2004).

Observations of infant behavior in the SSP allow for classification of infant behavior patterns into secure, insecure-avoidant, and insecure-resistant strategies. A secure (B) child seeks contact with the parent upon reunion, either physically or by distance interaction, to be comforted or reassured after the separation and resume exploration of the environment when he/she is settled. Insecure-avoidant (A) children, on the other hand, focus on the environment at the moment of reunion, ignoring the parent or even turning away from the parent. The reunion behavior of an insecure-resistant (C) child is characterized by anxious contact seeking and clinging and at the same time resisting contact with the parent. Resistant children are usually clearly distressed and their interaction with the parent may have an angry quality. On top of these classifications, the level of disorganization can be determined. Disorganized (D) children show a temporary breakdown of their secure, avoidant or resistant strategy of dealing with the return of the parent after separation, for example by simultaneous display of contradictory behaviors such as distress and avoidance (Main & Solomon, 1990).

Cortisol is released as a result of many aspects of an organism's interaction with the environment, including response to novelty and psychological stressors (Gunnar, 1994; Kirschbaum & Hellhammer, 1989; 1994). In normal situations, production of cortisol follows a diurnal rhythm with high levels at awakening, an increase in secretion shortly after awakening, followed by a decline throughout the day (Kirschbaum & Hellhammer, 1989; Watamura, Donzella, Kertes, & Gunnar, 2004). This diurnal rhythm in basal cortisol levels is relatively stable in adults, but early in life the Hypothalamic-Pituitary-Adrenal (HPA) system shows instability, and it continues to mature throughout infancy and childhood (De Weerth & Van Geert, 2002; De Weerth, Zijl, & Buitelaar, 2003; Watamura et al., 2004).

In stressful conditions, cortisol levels may rise. Cortisol response to stress serves an important function in adaptation to novel or stressful circumstances (Gunnar & Donzella, 2002; Van Bakel & Riksen-Walraven, 2004). Various studies have tested the effect of stressful events on HPA-axis functioning in infants, most of them focusing on cortisol levels around the SSP as related to infant attachment classification. Several non-clinical studies on physiological reactions to the SSP documented children's tendency to show elevated cortisol levels after the procedure. The most consistent finding is that no or only little adrenocortical activation is observed in securely attached infants (Gunnar, Brodersen, Nachmias, Buss, & Rigatuso, 1996, Spangler & Grossmann, 1993). Several studies reported increases in cortisol levels for the disorganized infants (Hertsgaard et al., 1995; Spangler & Grossmann, 1993; Spangler & Schieche, 1998).

Results for both insecure-avoidant and insecure-resistant groups are equivocal. In some studies, both insecure groups were found to have raised cortisol levels after the SSP (Spangler & Grossmann, 1993), others found increased cortisol levels only for insecure-resistant children (Spangler & Schieche, 1998). Spangler and Schieche (1998) interpreted their findings for the insecure-resistant group

as supporting an arousal model, assuming associations between behavioral and physiological activation during stress. This model implies that temperamental factors are possibly involved in the physiology of attachment. For example, as found by Gunnar and Donzella (2002), more reactive and irritable children display higher levels of cortisol when faced with a stressor, especially when they have an insecure attachment relationship. However, the aforementioned studies involved relatively small samples, and larger samples with substantial numbers of children in each of the attachment classification groups are needed to draw firmer conclusions on the association between attachment and cortisol reactivity and diurnal rhythm.

Until now, studies have only investigated attachment in relation to stress reactivity, neglecting the relation between attachment and infant diurnal rhythm of cortisol excretion (but see Adam & Gunnar, 2001, for diurnal rhythm and attachment status in adults). However, differences in cortisol reactivity for the different attachment categories may be related to systematic differences in slope of their diurnal rhythms. Although considerable intra- and inter-individual variation is found in cortisol diurnal rhythm in young infants (De Weerth & Van Geert, 2002) some stability after the first birthday has been suggested (Larson, White, Cochran, Donzella, & Gunnar, 1998) and is in fact presumed in studies on cortisol reactivity in the SSP. In the current study diurnal cortisol rhythm is assessed and related to infant attachment classification.

Parental depression may negatively influence infants' physiological regulation. More specifically, in several studies maternal depression was related to higher cortisol levels in infants, which could indicate both environmental and biological mechanisms of transmission (Ashman, Dawson, Panagiotides, Yamada, & Wilkinson, 2002; Essex, Klein, Cho, & Kalin, 2002; Halligan, Herbert, Goodyer, & Murray, 2004; Lupien, King, Meaney, & McEwen, 2000; Young, Vazquez, Jiang, & Pfeffer, 2006). Maternal depression has also been associated with attachment quality. Depression is thought to compromise sensitive parenting behavior, which in turn can undermine the development of a secure attachment relationship. However, the empirical evidence for this association is not unequivocal (see Cummings & Davies, 1994). Research on severe and chronic depression, as well as studies with clinical samples showed a significant association between maternal depression and attachment insecurity (e.g. Teti, Gelfand, Messinger, & Isabella, 1995). In community-based samples, however, the effect of maternal depressive symptoms on attachment quality is less clear; meta-analyses reported small or even insignificant effect sizes (Atkinson et al., 2000; Van IJzendoorn, Schuengel & Bakermans-Kranenburg, 1999). Other studies suggested that pre- and postnatal depression might influence mother-child interaction (Lundy et al., 1999; Righetti-Veltema, Bousquet & Manzano, 2003).

In the current study we examine both cortisol reactivity to a stressor and the diurnal rhythm of cortisol in relation to infants' attachment status. We expect

higher stress reactivity in insecurely attached children than in securely attached children. Furthermore, we expect that infants in the disorganized group differ in their cortisol reactivity from non-disorganized infants. With respect to diurnal rhythm, we expect to find a general pattern with higher morning than evening cortisol values. Since this study is the first to explore cortisol diurnal rhythm in relation to infant attachment status, we have no directed hypothesis on differences among attachment groups. Furthermore, we examine the moderating role of maternal depression on the association between attachment quality and cortisol levels. As maternal depression is related to insecure infant attachment and sub-optimal cortisol outcomes, maternal depression is hypothesized to act as an additional risk factor in the relation between insecure attachment and cortisol.

Method

Setting

The current investigation is embedded within the Generation R Study, a prospective cohort study investigating growth, development and health from fetal life into young adulthood in Rotterdam, the Netherlands, which has been described in detail elsewhere (Jaddoe et al., 2007; 2008). In the Generation R Study, we obtained detailed measurements of the child's development in a rather homogeneous subgroup: The Generation R Focus Study. Only children of Dutch national origin were included in this group, meaning that the children, their parents and their grandparents were all born in the Netherlands. The participating children were born between February 2003 and August 2005. The children visited the research center regularly for various somatic and behavioral assessments. Written informed consent was obtained from all participants. The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam.

Study population

In the current investigation, data are presented of the 14-month visit of the Generation R Focus Study. A total of 882 infants and their parents participated between June 2004 and November 2006. In the first part of the visit, that lasted about 30 minutes, anthropometric and physiological measurements were conducted. Then, the Strange Situation Procedure (SSP) was administered, followed by assessments of the infants' motor functioning. In the SSP, twenty-four parents participated with two children (on different days). One child of each sibling pair was randomly excluded to avoid bias due to paired data. Another 29 children were excluded because of technical or procedural problems during the SSP. Of the remaining children, another 108 were not eligible for analysis because they completed the SSP with their fathers. After exclusion of these children, the study population consisted of 721 mother-infant dyads. Of this group, we had complete data on cortisol reactivity for 369 children, and 363 children were included in one or more measures of cortisol diurnal rhythm. Reasons for non-

response were lack of time and failure to obtain saliva samples. A high rate of refusal to chew on cotton swabs is not uncommon in this age group and has been reported before (Goldberg et al., 2003). This is typically found in infants that are not familiar with pacifiers. A non-response analysis was conducted to check for differences between children with and without cortisol data. For cortisol diurnal rhythm, differences between the groups were found for age at assessment, ($p <$ .05) gender ($p < .01$), and breastfeeding at the age of six months ($p < .05$). The group with diurnal cortisol data consisted of younger children, more boys, and the children were breastfed more often at six months of age. For cortisol stress reactivity, the groups differed on age at assessment ($p < .05$) and gender ($p < .01$); again, these children were younger and there were more boys in the group for which the data was available. For both cortisol reactivity and diurnal rhythm, non-response analyses did not show differences on maternal depression. Information about lifetime depression was available for 627 mothers.

Procedures and measures

Strange Situation Procedure. Parent-infant dyads were observed in the Strange Situation Procedure (SSP) when the infant was about 14 months of age (M = 14.7 SD = 0.9). The SSP is a widely used and well-validated procedure to measure the quality of the attachment relationship. The procedure consists of 7 episodes of 3 minutes each and is designed to evoke mild stress in the infant to trigger attachment behavior evoked by the unfamiliar lab environment, a female stranger entering the room and engaging with the infant, and the parent leaving the room twice (see Ainsworth et al., 1978, for the protocol). The SSP used in the current study included all these stimuli but to make it fit into a tight time schedule, we shortened the (pre-)separation episodes with one minute keeping the critical reunion episodes intact. Attachment behavior was coded from DVD-recordings according to the Ainsworth et al. (1978) and Main and Solomon (1990) coding systems by two reliable coders, trained at the University of Minnesota. Inter-coder agreement was calculated on 70 SSPs that were coded by both coders. For ABCD classification, inter-coder agreement was 77% ($\kappa = .63$); agreement on disorganization was 87% ($\kappa = .64$). 8% of the cases were discussed with one of two expert coders and classification was assigned after consensus was reached.

Salivary cortisol: diurnal rhythm and stress reactivity. Prior to the 14-month visit of the Generation R Focus Study parents were asked to collect saliva samples from their child at home using Salivette sampling devices (Sarstedt, Rommelsdorf, Germany). Parents received detailed written instructions with pictures concerning the saliva sampling. They were asked to collect five saliva samples during one single weekday at home: immediately after awakening, 30 minutes later, between 11 am and 12 pm, between 3 and 4 pm, and at bedtime; and to note down the sampling times. The child was supposed not to eat or drink 30 minutes before each sampling. The children were otherwise free to follow their normal daily

routines on the sampling day. Parents were asked to keep the samples stored in a freezer until they visited the research centre. If parents forgot to bring the samples, they were asked to send the Salivettes by postal mail. For 397 children (55%) one or more home saliva samples were returned. One child was excluded because it was older than 20 months. To compute a cortisol composite measure, at least the first sample and, depending on the measure, one or two subsequent samples had to be obtained, which left 363 children for the diurnal assessments. None of the children used systemic corticosteroid medication, but 12 children used other corticosteroid-containing medication. Excluding these children did not change the results, so they were included in further analyses. During the visit at the research centre at 14 months of age, three saliva samples were taken; the first prior to the SSP, the second directly after the SSP (which was on average 10 minutes after the first separation of the SSP) and the third about 15 minutes later (M = 16.3, SD = 8.3). For 369 children (51%) three samples were obtained.

Samples were centrifuged and frozen at -80°C. After completion of the data collection, all samples were sent in one batch (frozen, by courier) to the Kirschbaum laboratory (Technical University of Dresden, Biological Psychology, Professor Dr. Kirschbaum) for analysis. Salivary cortisol concentrations were measured using a commercial immunoassay with chemiluminescence detection (CLIA; IBL Hamburg, Germany). Intra- and interassay coefficients of variation were below 7% and 9%, respectively. For each time point, cortisol values that were above the 99th percentile (>200 nmol/L) were excluded (n = 12) from the analysis to reduce the impact of outliers.

Cortisol analyses. The daytime profile of cortisol secretion was characterized by calculating composite variables of the separate cortisol measurements. In this way we took into account the relation between the separate cortisol values within each child. We determined the area under the curve with respect to ground (AUC_G), which is a measure of total cortisol secretion during the day (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). The AUC_G was established by calculating the total area under the curve from the cortisol measurements in nmol/L on the y-axis and the time between the cortisol measurements on the x-axis. This takes into account the difference between the single measurements from each other and the distance of these measures from the ground, or zero (Pruessner et al. 2003, p. 918). To correct for differences in length of day, the AUC_G was divided by number of hours between the first cortisol measurement (at awakening) and the last cortisol measurement (before going to bed) (see Watamura et al., 2004). Sleeping hours during the day were not associated with this composite measure. The AUC_G was computed only for children having at least three saliva samples (N = 228). The cortisol awakening response (CAR) was used as an index of HPA axis activity. It was calculated as the difference between cortisol value at awakening and the value 30 minutes after awakening (Kunz-Ebrecht, Kirschbaum, Marmot, & Steptoe, 2004). For CAR, data was available for

N = 258 children. As a measure of the diurnal cortisol decline we calculated the slope by fitting a linear regression line for each child, which predicted the cortisol values from time since awakening. The slope was computed by using the first and last saliva samples and at least one other cortisol measurement. To avoid any effect of the CAR on the slope (Adam, Hawkley, Kudielka, & Cacioppo, 2006; Cohen, Schwartz, Epel, Kirschbaum, Sidney, & Seeman, 2006), the second cortisol sample (30 minutes after awakening) was not included in this measure of the slope. Data were available for N = 248 children. These composite measures of cortisol were moderately intercorrelated (AUC_G-CAR: r =.22, p < .01; AUC_G-slope: r = -.23, p < .01; CAR-slope: r = .51, p < .01).

For stress reactivity a delta was calculated between the last sample ($cortisol_{postSSP}$) and the first sample ($cortisol_{preSSP}$). The second assessment, just after the SSP, was not used, as it was too close to the onset of stress to show an increase. To control for the Law of Initial Values (LIV; Wilder, 1968), which states that the direction of response of a body function depends to a large degree on the initial level of that function, in subsequent analyses this delta was adjusted for the first sample.

Maternal lifetime depression. The Composite International Diagnostic Interview (WHO, 1990) version 2.1 was conducted during a home-visit at 30 weeks of pregnancy to assess lifetime prevalence of psychiatric disorders in the pregnant women. The CIDI is based on the definitions and criteria of the DSM IV; good to excellent psychometric properties have been reported (Andrews & Peters, 1998; Wittchen, 1994). Interviewers had been trained at a WHO training center. The mother's partner was not present during the interview. In the current study we used lifetime diagnoses of unipolar depressive disorder. Unipolar depressive disorder was defined as diagnoses of dysthymia, a single episode of major depression (mild, moderate or severe) and recurrent major depression (mild, moderate or severe).

Results

Attachment

The distribution of the attachment classifications was as follows: 57.8% secure (n = 413), 19.0% avoidant (n = 136), 23.2% resistant (n = 166). Of all children, 22.5% were classified as disorganized (n = 162), 77.5% were non-disorganized (n = 559). No differences were found between the distribution of the complete group (N = 721), and the group for which data on cortisol reactivity or cortisol diurnal rhythm was available (respectively χ^2 (3, N = 721) = 4.11, p = .25; χ^2 (3, N = 721) = 4.15, p = .25).

Table 1 shows the demographic variables for attachment security. No overall differences were found, except for parity. Avoidant children were more often the first child, χ^2 (2, N = 714) = 12.87, p < .01. In Table 2, demographics are shown according to disorganization status. Mothers of non-disorganized children consumed more alcohol during the period they breastfed, χ^2 (1, N = 490) = 5.32, p < .05. Some demographic variables were related to the cortisol measures; age at

Table 1. *Child and parent characteristics of the secure, insecure-avoidant, and insecure-resistant attachment groups*

	Secure (n = 413)	Insecure-avoidant (n = 136)	Insecure-resistant (n = 166)
Child characteristics			
Gender, % female	49.6	42.6	52.1
Parity, % firstborn	59.2	75.7	59.0**
Age at 14 months visit	14.6 (0.9)	14.8 (1.1)	14.7 (0.9)
Time of assessment cortisol$_{preSSP}$	11:28 (1:58)	11:31 (1:57)	11:28 (2:07)
Parental characteristics			
Age at intake mother	31.6 (3.9)	31.7 (3.6)	32.4 (3.7)
Maternal educational level, % low/medium	35.9	35.3	37.0
Marital status, % single	5.7	5.3	1.3
Smoking during pregnancy, %	12.4	14.2	8.0
Alcohol during pregnancy, %	56.4	48.9	61.3
Alcohol during breastfeeding, %	64.2	70.7	58.6

Note. Unless otherwise indicated, values are *M* and (*SD*). ** $p < .01$

Table 2. *Child and parent characteristics of the disorganized and non-disorganized attachment groups*

	Disorganized (n = 162)	Non-Disorganized (n = 559)
Child characteristics		
Gender, % female	51.2	48.1
Parity, % firstborn	58.6	63.3
Age at 14 months visit	14.6 (0.9)	14.7 (0.9)
Time of assessment cortisol$_{preSSP}$	11:06 (1:59)	11:34 (1:59)
Parental characteristics		
Age at intake mother	32.0 (3.8)	31.8 (3.8)
Maternal educational level, % low/medium	33.1	36.8
Marital status, % single	3.8	4.7
Smoking during pregnancy, %	9.3	12.7
Alcohol during pregnancy, %	51.9	57.5
Alcohol during breastfeeding, %	54.5	66.4*

Note. Unless otherwise indicated, values are *M* and (*SD*). * $p < .05$

14-months visit was related to slope ($r = .16, p < .05$), and smoking during pregnancy was related to CAR (F (2, 229) = 3.03, p = .05). Time of cortisol assessment was not related to cortisol measures or attachment classification, in fact, none of the demographic variables were related to both cortisol and attachment measures. Maternal lifetime depression was not related to attachment security, F (2, 618) = 0.96, p = .39, nor to disorganization status, F (1, 625) = 0.14, p = .71.

Attachment and cortisol stress reactivity

To test whether cortisol stress reactivity differed across attachment classifications, an ANCOVA was performed. Because attachment security and attachment disorganization are considered orthogonal dimensions (Van IJzendoorn et al., 1999), they were entered as two separate factors. Maternal lifetime depression was entered as a covariate, as was the first cortisol assessment to control for initial cortisol values. We found a main effect for attachment security, F (2, 308) = 9.03, $p < .01$, $\eta^2 = .06$. Resistant children differed from all other groups, displaying larger deltas, meaning larger differences between pre- and post-stressor assessment (post hoc analysis using Bonferroni criterion; $p < .01$), see Figure 1. In analyses, difference scores for cortisol (deltas) were used. In order to enhance interpretation, in Figure 1 cortisol values are shown. We did not find significant differences in stress reactivity between the disorganized group and the non-disorganized group. No main effect was found for maternal lifetime depression. A significant interaction effect was found for attachment security and maternal depression (F (2, 308) = 4.22, $p < .05$, $\eta^2 = .03$).

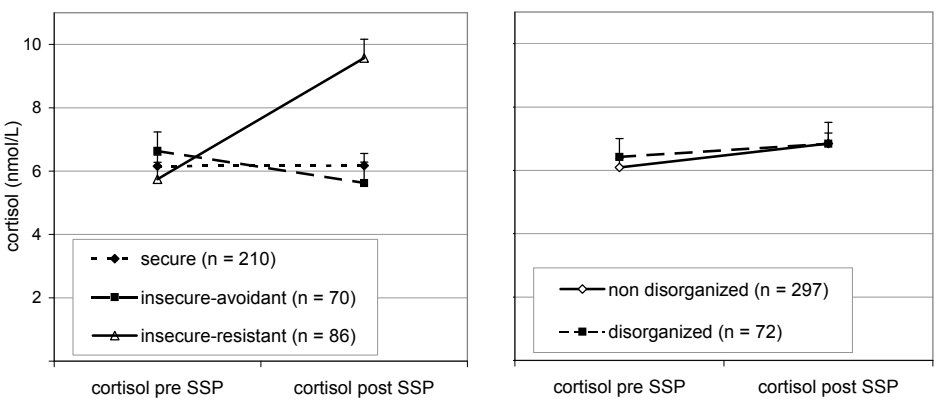

Figure 1. *Insecure-resistant children show high cortisol reactivity compared to the other groups; no differences in cortisol reactivity between disorganized and non-disorganized children*

Locating the interaction effect, we found that resistantly attached infants showed highest cortisol reactivity, in particular when their mothers scored high on depression (r (79) = .21, p (one-tailed) < .05, see Figure 2). In a separate ANOVA, we found no differences in cortisol levels between the groups prior to the SSP (attachment security: p = .53; attachment disorganization: p = .61). When the middle cortisol assessment was aggregated with the first cortisol assessment as a baseline level, similar outcomes were obtained (data not shown).

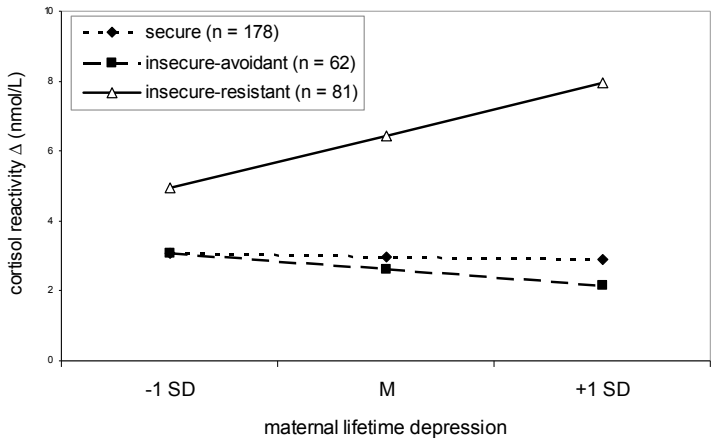

Figure 2. *Stronger effect of maternal lifetime depression on cortisol reactivity of insecure-resistant children compared to insecure-avoidant and secure children*

Attachment and cortisol diurnal rhythm

The excretion of cortisol did show the expected diurnal pattern, with high levels at awaking and a decline throughout the day. In the cortisol diurnal curves of the infants, most children showed no morning rise. We performed an ANCOVA to test the effect of attachment quality on the cortisol measures AUC_G, slope, and CAR. Again, attachment security and attachment disorganization were entered as factors, and maternal depression was included as a covariate. A main effect of disorganization was found for slope (F (1, 213) = 3.99, p < .01, η^2 = .03), indicating a more flattened slope for children with a disorganized attachment classification (slope disorganized group = -0.84, SE = 0.11; slope non-disorganized group = -1.16, SE = 0.06; Figure 3). Also, for AUC_G, an interaction effect was found for attachment security and disorganization, (F (2, 195) = 3.34, p = .04, η^2 = .03). Disorganized-secure infants showed higher cortisol excretion (AUC_G = 10.49, SE = 1.27) than disorganized-insecure infants (AUC_G = 7.48, SE = 1.27 for children with a secondary avoidant classification, and AUC_G = 7.66, SE = 1.05 for children with a secondary resistant classification). Compared to the non-disorganized group, cortisol excretion in the disorganized group was more divergent, dependent on the second classification. No effects were found for CAR or maternal lifetime depression.

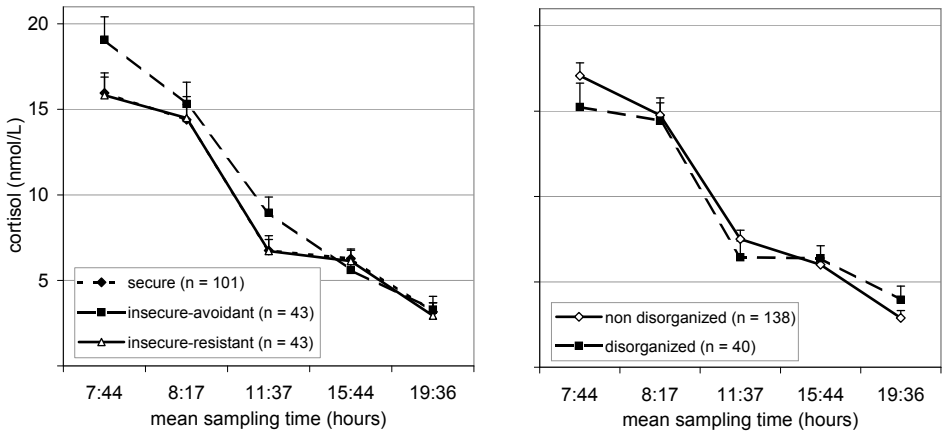

Figure 3. *No differences in cortisol diurnal rhythm for secure, insecure-avoidant, and insecure-resistant children; flattened slope for disorganized children compared to non-disorganized children*

Discussion

In a large cohort study with pertinent data on 369 mother-infant dyads, we found that infant attachment quality was related to cortisol stress reactivity, as assessed before and after the SSP. Resistant infants differed from all other groups, showing the largest increase in cortisol excretion after the SSP. Cortisol diurnal rhythm showed the expected diurnal pattern, with disorganized infants displaying a more flattened slope than non-disorganized infants. Maternal lifetime depression appeared to be a risk factor further elevating cortisol reactivity in infants with a resistant attachment relationship.

Cortisol reactivity and insecure-resistant attachment

Infants with a resistant attachment relationship showed the largest difference between pre and post SSP cortisol assessments compared to all other groups. This result converges partly with the outcomes of previous studies. Resistant infants were found to show higher cortisol levels after a stressful stimulus in some previous studies (Spangler & Schieche, 1998), but not in others (Gunnar et al., 1989; Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996). In our study, infants classified as disorganized did not show increased reactivity, contrary to some of the previously reported results (Hertsgaard et al., 1995; Spangler & Grossmann, 1993). It may be the case that in previous studies reporting high reactivity in disorganized infants (Hertsgaard et al., 1995; Spangler & Grossmann, 1993) the majority of the infants had a secondary *resistant* classification; meta-analytic evidence confirms the suggestion that resistant infants have a strongly elevated chance of becoming classified as disorganized (Van IJzendoorn et al., 1999).

According to Weinfield, Sroufe, Egeland and Carlson (2008), resistant infants' history of erratic responsiveness renders them less able to direct attachment behaviors at caregivers when appropriate. Their 'maximizing' strategy might result in more physiological arousal than the 'minimizing' strategy of avoidant infants. Spangler and Schieche (1998) also proposed that resistant infants' high activation of the attachment system could not be terminated because they were not able to use the attachment figure effectively. Resistant infants 'maximize' their attachment behavior while they are at the same time unable to find a state of homeostasis in interaction with their caregiver (Cassidy & Berlin, 1994).

In contrast to the resistant infants, infants with secure or avoidant attachment classifications did not show significant increases in cortisol levels. This is partly convergent with previous literature. Both Hertsgaard et al. (1995) and Spangler and Schieche (1998) did not find increases in cortisol in avoidant infants. Minimizing the display of negative emotions might protect avoidant infants against elevated physiological reactivity in mildly stressful settings. Securely attached infants showed hardly any heightened cortisol responses in previous studies. They exhibit appropriate behavioral strategies in coping with the separation (Spangler & Schieche, 1998). According to Bowlby (1973, p. 150), these behavioral strategies can be regarded as an 'outer ring' of life-maintaining systems. When this 'outer ring' is in homeostasis, an adaptation of the 'inner ring', or physiological system, is not necessary.

Another, complementary, explanation can be found in temperamental characteristics of the infant. The concept of regulation plays a central role in both attachment and temperament theory (Vaughn, Bost, & Van IJzendoorn, 2008). Temperamental characteristics of the infants have been found to play a role in stress physiology (e.g., Dettling, Parker, Lane, Sebanc, & Gunnar, 2000). In addition, previous studies documented the association between lowered temperamental reactivity in avoidant children, and heightened temperamental reactivity in resistant children (Vaughn et al., 2008). Interpreting our finding of elevated cortisol reactivity in resistant but not in avoidant children, we speculate that the dual risk of temperamental reactivity and an insecure-resistant attachment relationship may be responsible for the increased cortisol secretion after stress in resistant children. Avoidant infants are supposed to be buffered against elevated cortisol reactivity to mild stress because of their less reactive and somewhat more aloof temperament.

Diurnal rhythm and disorganization

Daytime cortisol showed the expected diurnal pattern, with higher levels at awakening and lower levels at the end of the day (e.g. Mantagos, Moustogiannis, & Vagenakis, 1998; Price, Close, & Fielding, 1983; Spangler, 1991). However, De Weerth and Van Geert (2002) state that while at group level there is evidence for the presence of a diurnal rhythm of cortisol from the early age of 2 months, individuals

can vary greatly in the age at which they acquire the rhythm, which according to Gunnar and Donzella (2002) can be up to 4 years of age. To our knowledge, no previous studies related attachment quality to cortisol diurnal rhythm. In the current study, disorganized infants showed a more flattened slope of the diurnal rhythm than non-disorganized children. A flattened daytime pattern of cortisol –in its extreme form hypocortisolism– has often been found among children growing up in orphanages with structural neglect of basic emotional needs (see Gunnar & Vazquez, 2001, for a review). As a disorganized attachment relationship is thought to originate from extremely insensitive or even frightening parenting, this may cause similar physiological dysregulation in the disorganized group. Furthermore, higher diurnal cortisol excretion was found for disorganized-secure infants, whilst disorganized-insecure groups showed lower cortisol excretion. The interaction effect might indicate the intricate nature of these sub-groups. Cortisol excretion in children with a secondary insecure classification might be decreased in order to prevent enduring activation of the HPA-axis, whereas a secondary secure classification may indicate differential activation of the infants' endocrinological system, causing higher levels of excretion. Replications are essential to confirm these outcomes as our study is the first to be able to differentiate between these sub-groups.

Cortisol reactivity and maternal depression

Although several studies report maternal depression to affect both diurnal and reactivity cortisol levels in offspring (Azar, Paquette, Zoccolillo, Baltzer, & Tremblay, 2007; Brennan et al., 2008; Lupien et al., 2000; Young et al., 2006), in our study involving a non-clinical population such main effects were not found. Nevertheless, a clear interaction effect was found: infants with a resistant attachment relationship and a depressed mother displayed the strongest cortisol reactivity. The interaction between depression and attachment insecurity suggests a double risk model. In the case of resistant infants, the uncertainty about the mothers' availability is suggested to be associated with heightened attachment behavior, increasing the infant's monitoring of the caregiver and decreasing the exploratory competence (Cassidy & Berlin, 1994). In addition, infants of depressed mothers were found to experience reduced sensitivity and increased intrusiveness in interaction with their mothers (Goodman & Gotlib, 1999). Resistant attachment and maternal depression appear to compromise physiological regulation in an additive fashion.

Limitations

Some limitations of the current study need to be discussed. First, the Generation R Focus Study is a relatively homogeneous sample. However, the use of a homogeneous sample may have only led to an underestimation, and not an overestimation of the effects. Second, cortisol was sampled at 14 months of age.

Cortisol levels at this age do show some intra- individual instability (De Weerth & Van Geert, 2002). However, data on the development of cortisol secretion throughout infancy and childhood are scarce, and we did find evidence for an established pattern. Again, instability may have led to an underestimation of the differences among attachment groups. Third, a relatively large part of the participants could not be included in cortisol analyses, due to various reasons. Clearly informing parents about sampling could help to gain more and better saliva samples, however, sampling might remain difficult in 14-month olds. Lastly, a slightly shortened version of the SSP was used, in order to make it fit into the schedule of the visit. This minimal procedural change did not appear to modify the stress of the SSP, since the number of infants for whom the situation appears to be most stressful (resistant and disorganized classifications) was not lower in the current study compared to the standard distribution.

Conclusion

We documented the vulnerability of resistant infants in physiological stress regulation, especially in combination with care from a mother with a lifetime diagnosis of depression. Because of their small numbers in most attachment studies, resistant infants have been understudied as a separate insecure group. Our finding of elevated physiological stress reactivity in resistant children makes clear that this group can and should be differentiated from the other insecure attachment groups. We also showed that disorganized infants differed from non-disorganized infants in their diurnal cortisol rhythm, as they displayed a more flattened daily curve. This finding stresses the disturbed nature of disorganized attachments as one of the most important risks for developmental psychopathology. Our large-sample study suggests the differential physiological concomitants of avoidant, resistant, and disorganized attachments. Because infant attachment patterns have been shown to be relatively stable in stable environments (Fraley, 2002) insecure attachments may have long-term consequences for mental health, in particular in combination with other risk factors such as parental depression. Here we found that insecure-resistant and disorganized attachments can go 'under the skin' and may lead to deviating cortisol reactivity and daily patterns. From a biological perspective (Sapolsky, 2004) adverse early experiences can make humans and other animals more prone to stress and stress-related diseases, and attachment relationships may mediate the intergenerational transmission (Meaney, 2001) of this elevated vulnerability to emotional dysregulation.