Infant attachment and stress regulation : a neurobiological study

Luijk, P.C.M.

4 The association between parenting and attachment is moderated by a polymorphism in the mineralocorticoid receptor gene: Evidence for differential susceptibility

Maartje P. C. M. Luijk, Anne Tharner, Marinus H. van IJzendoorn, Marian J. Bakermans-Kranenburg, Vincent W.V. Jaddoe, Albert Hofman, Frank C. Verhulst, and Henning Tiemeier.
Manuscript submitted for publication

Abstract
Maternal sensitive responsiveness and extreme insensitivity only partly explain the variance in attachment security. Differences in attachment security may well be rooted in the interplay of genetic variations and environmental factors. The association between parenting (observed sensitive responsiveness and extreme insensitivity) and attachment security (assessed with the Strange Situation Procedure) was hypothesized to be moderated by genes involved in the regulation of the stress response: the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) genes. A significant GxE interaction was found: Infants carrying the minor MR allele (G) were significantly more securely attached if their mothers showed more sensitive responsiveness, *and* significantly less securely attached if their mothers showed more extremely insensitive behaviors. These associations were not significant for carriers of the AA genotype of MR. Findings are discussed from a differential susceptibility perspective.

Introduction
Attachment security is a developmental milestone, defined as the child's need to seek proximity to and comfort from a potentially protective caregiver in times of stress (e.g., illness, danger, Bowlby, 1969/1982). Maternal sensitive responsiveness to the baby's stress and distress signals is considered to be an important determinant of attachment security (Ainsworth, Blehar, Waters, & Wall, 1978; Bakermans-Kranenburg, Van IJzendoorn & Juffer, 2003), whereas extreme insensitivity bordering on neglectful parenting, elevates the risk for insecure attachment (Out, Bakermans-Kranenburg & Van IJzendoorn, 2009).

Maternal sensitive responsiveness and extreme insensitivity only partly explain the variance in attachment security, and attachment differences may also be rooted in genetic differences. Main effects of genetic factors on attachment

security have been found to be elusive, both in behavioral and molecular genetic studies (Bakermans-Kranenburg & Van IJzendoorn, 2007; Bokhorst et al., 2003; O'Connor & Croft, 2001; Roisman & Fraley, 2008). However, genetic effects on child development are probably hidden in interactions with environmental factors (Barry, Kochanska & Philibert, 2008; Belsky et al., 2009).

Because attachment is functional for the regulation of stress (Bowlby, 1969/1982; Hertsgaard, Gunnar, Erickson & Nachmias, 1995), we focus on genes involved in the regulation of the stress response: the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) that have been implicated in the variability of HPA axis responses to social stressors (DeRijk & De Kloet, 2008). We hypothesize that polymorphisms in GR and in MR are important candidates for GxE in the case of attachment security. Following the concept of differential susceptibility as a specific type of GxE interaction (Belsky, Bakermans-Kranenburg & Van IJzendoorn, 2007), we hypothesize that a combination of receiving more responsive parenting and carrying minor alleles of GR or MR leads to a more *secure* attachment relationship, whilst a combination of experiencing more extremely insensitive parenting and carrying minor alleles of GR and MR is related to a more *insecure* attachment relationship.

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 (Jaddoe et al., 2007; 2008). In the Generation R Study, detailed measurements of the child's development in an ethnically homogeneous subgroup were obtained. 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

DNA was collected from cord blood samples at birth. At the age of 14 months, infants and their mothers participated in the Strange Situation Procedure (SSP). For 601 infants, information on MR and GR genotypes and quality of attachment was available. Within this group, maternal sensitive responsiveness was observed for 530 children, maternal extreme insensitivity was observed for 543 children. Participant characteristics are displayed in Table 1. A non-response analysis was conducted to check for differences between children with and without sensitivity data. Differences between the groups were found for gestational age ($p < .05$), family income ($p < .05$), and maternal alcohol use during pregnancy ($p < .05$). No differences were found for attachment security or genotype ($.08 < p < .92$). Of the demographic variables, only parity was related to maternal sensitivity, genotype, and attachment security at the same time. Taking parity into account in the analyses did not change the results.

Table 1. *Sample characteristics*

Child characteristics	
Child gender, % female	48.8
Parity, % firstborn	62.1
Birth weight in grams	3507 (540)
Gestational age in weeks	40.0 (1.7)
Apgar score	8.6 (1.1)
Richters Security Score	0.2 (2.6)
Parental characteristics	
Age at intake mother	31.8 (3.8)
Maternal educational level, % low/medium	35.6
Hours working, mother	28.3 (12.4)
Marital status, % single	4.4
Smoking during pregnancy, %	11.0
Alcohol during pregnancy, %	54.2
Breastfeeding at 6 months, %	29.5
Maternal Sensitive Responsiveness	6.6 (1.2)
Maternal Extreme Insensitivity	1.3 (1.0)

Note. Unless otherwise indicated, values are mean (SD).

Procedures and measures

Strange Situation Procedure. Parent-infant dyads were observed in the Strange Situation Procedure (SSP, Ainsworth et al., 1978) when the infant was 14.7 months of age (*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 seven 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. Attachment behaviors may be categorized as secure or insecure. When stressed, secure infants seek comfort from their mothers, which proves effective, enabling the infant to return to play. Insecure-avoidant infants show little overt distress, while turning away from or ignoring mother on reunion. Insecure-resistant infants are distressed and angry,

but ambivalent about contact, which does not effectively comfort and allow the child to return to play. Distribution of attachment classifications was as follows: 57.3% secure (n = 413), 18.9% insecure-avoidant (n = 136), 23.0% insecure-resistant (n = 166). No classification could be assigned for n = 6 (0.8%) children. Inter-coder agreement was calculated on 70 SSPs that were coded by both coders, inter-coder agreement was 77% (κ = .63). Eight percent of the cases were discussed with one of two expert coders and classification was assigned after consensus was reached. Continuous scores for attachment security were computed using Van IJzendoorn and Kroonenberg's (1990) adaptation of Richter's algorithm (Richters, Waters & Vaughn, 1988).

Maternal sensitive responsiveness. Maternal sensitive responsiveness was observed during a psychophysiological assessment in the 14 months lab visit with Ainsworth's rating scales for sensitivity (Ainsworth, Bell, & Stayton, 1974). Scores for sensitive responsiveness were based on the subscale scores for sensitivity and cooperation (r = .87), both scored on 9-point rating scales with higher scores indicating more sensitive responsiveness. The intraclass correlation for sensitive responsiveness (single measure, absolute agreement) was .65 (n = 82).

Maternal extreme insensitivity. Maternal extreme insensitivity was observed during the 14 months lab visit, by coders unaware of the ratings of maternal sensitivity and attachment security. The scale includes 1) parental withdrawal and neglect; and 2) intrusive, negative, aggressive or otherwise harsh parental behaviors (Out, et al., 2009). Discrete extremely insensitive behaviors were coded on a 9-point scale, with higher scores indicating more extreme insensitivity. The intraclass correlation (single measure, absolute agreement) was .63 (n = 36).

Genotyping. DNA was collected from cord blood samples at birth. Participants were genotyped for polymorphisms in the glucocorticoid receptor gene, *Bcl*I (rs41423247), *TthIII*I (rs10052957), GR-9β (rs6198), N363S (rs6195) and ER22/23EK (rs6189 and 6190); and the mineralocorticoid receptor gene (rs5522). To check for potential contamination with maternal blood, gender was determined in male participants. Contamination occurred in < 1% of cases, which were excluded. Genotyping was performed using Taqman allelic discrimination assay (Applied Biosystems, Foster City, CA) and Abgene QPCR ROX mix (Abgene, Hamburg Germany). The genotyping reaction was amplified using the GeneAmp® PCR system 9600 (95° C (15 min), then 40 cycles of 94° C (15 s) and 60° C (1 min)). The fluorescence was detected on the 7900HT Fast Real-Time PCR System (Applied Biosystems) and individual genotypes were determined using SDS software (version 2.3, Applied Biosystems). Genotyping was successful in 97-99% of the samples. To confirm the accuracy of the genotyping results 276 randomly selected samples were genotyped for a second time with the same method. The error rate was less than 1% for all genotypes. For the glucocorticoid receptor gene we used the genotype data for each of the 5 polymorphisms to infer the haplotypes present in the population using the program PHASE, which

implements a Bayesian statistical method for reconstructing haplotypes from population genotype data (Stephens et al., 2001). For each haplotype, 3 genotype combinations were distinguished as carrying 0, 1, or 2 copies of the haplotype allele. The GR wildtype carries the major alleles of the polymorphisms. Genotype frequencies were in Hardy Weinberg equilibrium (χ^2s [1, N = 568 - 592] < 1.23, ps > .27). GR haplotypes and the MR SNP were not correlated. Due to low minor allele frequencies (3-5%), two haplotypes (N363S and ER22/23EK + GR-9β + *TthIII*I) were not used in further analyses. Table 2 shows the allele frequencies.

Table 2. *Distribution of GR haplotypes and MR and main effects on attachment security*

Haplotype / SNP	Allele frequency (%)[a]			MAF (%)	*r*	*p*
	0	1	2			
GR Wildtype	35	48	17	41	.04	.37
*Bcl*I	60	36	4	22	.05	.27
*TthIII*I + *Bcl*I	73	26	1	14	-.02	.61
GR-9β + *TthIII*I	76	23	1	13	-.06	.13
MR rs5522	79	19	2	11	.05	.20

[a] % of copies of the minor allele. MAF = minor allele frequency. All SNPs were in HWE (χ^2s [1, N = 568 - 592] < 1.23, ps > .27).

Results

GR haplotypes and the MR SNP were not related to attachment security (.13 < p < .61), maternal sensitive responsiveness (.59 < p < .99), or extreme insensitivity (.34 < p < .99). Maternal sensitive responsiveness and extreme insensitivity were only modestly correlated (r = -.14, p <.01). There were no main effects of sensitive responsiveness and extreme insensitivity on attachment security (r = .05, p = .18 and r = -.04, p = .38, respectively). Using a regression analysis, we tested for an interaction effect of maternal sensitive responsiveness, GR haplotypes, and MR on attachment security, controlling for extreme insensitivity. The same model was run for extreme insensitivity, controlling for sensitive responsiveness. In both models, main effects of GR haplotypes and MR did not reach significance (.06 < p < .86). The interaction between the MR SNP and sensitive responsiveness was significant (β = .10, p = .02), and a similar effect was found for the interaction between the MR SNP and extreme insensitivity (β = -.13, p = .005; Table 3). Interactions between GR haplotypes and maternal sensitive responsiveness were not significant (.34 < p < .99); the same was true for extreme insensitivity (.13 < p < .66). Locating the interaction effect, we found that infants carrying the minor MR allele (G) were significantly more securely attached if their mothers showed more sensitive responsive behaviors, and were significantly less securely attached if their mothers showed more extremely insensitive behaviors. These associations were not significant for carriers of the AA genotype of MR (Figure 1).

Table 3. *Regression analyses predicting attachment security from maternal sensitive responsiveness and extreme insensitivity and MR*

Sensitive Responsiveness	*B* (95% *CI*)	β	R^2	R^2_{change}	**Extreme Insensitivity**	*B* (95% *CI*)	β	R^2	R^2_{change}
Step 1			<0.01	<0.01	Step 1			<0.01	<0.01
Extreme insensitivity	-0.06 (-0.30; 0.18)	-0.02			Sensitive responsiveness	0.08 (-0.11; 0.27)	0.38		
Step 2			0.01	0.01	Step 2			0.01	0.01
Sensitive responsiveness	0.09 (-0.09; 0.28)	0.04			Extreme insensitivity	-0.07 (-0.31; 0.16)	-0.03		
MR	0.49 (-0.00; 0.98)	0.09			MR	0.48 (-0.01; 0.97)	0.09		
Step 3			0.02	0.01	Step 3			0.03	0.02
Sensitive responsiveness * MR	0.47 (0.07; 0.88)	0.10*			Extreme insensitivity * MR	-0.68 (-1.16;-0.21)	-0.13**		

Note. Final model for sensitive responsiveness: F (4, 497) = 2.47, $p < .05$, $R^2 = 2\%$; for extreme insensitivity: F (4, 497) = 3.13, $p < .05$, $R^2 = 3\%$. β is a standardized coefficient and denotes SD change in attachment security per SD change in the predictor. The statistics are derived from the final block of the regression models.

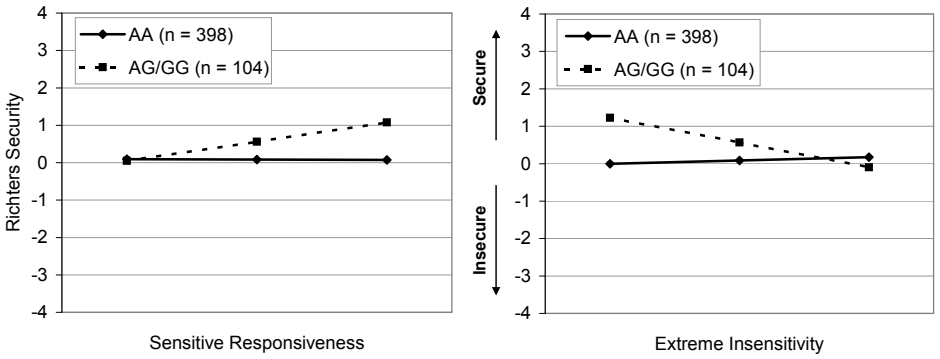

Figure 1. *Interaction between MR genotype and maternal sensitive responsiveness (left) and extreme insensitivity (right) on attachment security*

Discussion

Infants carrying the minor MR allele (G) were more securely attached if their mothers showed more sensitive responsiveness, *and* less securely attached if their mothers showed more extremely insensitive behaviors, whereas these associations were not significant for carriers of the AA genotype of MR. Genetic variation in MR thus seems to modulate infants' sensitivity to care, both in a positive (maternal sensitive responsiveness), as well as in a negative (maternal extreme insensitivity) environment. This supports the differential susceptibility hypothesis (Belsky et al., 2007).

MR is involved in the fast onset of responses and associated with processing of stressful information (DeRijk & De Kloet, 2008). We speculate that infants who are faster and better in processing information on maternal behaviors in stressful circumstances might be more susceptible to the effects of both positive care (sensitive responsiveness) and negative parenting (extreme insensitivity), for better *and* for worse. This potential mechanism should be examined in future biochemical as well as behavioral studies.

The two types of observed maternal behavior might be thought to reflect two extremes on a caregiving continuum. However, conceptually as well as statistically they indicate different, only weakly related dimensions of parenting. It should be noted that in the current, homogeneous middle class sample, quality of maternal care was not associated with attachment security. Generally, maternal care is only weakly to moderately associated with attachment, and null findings have also been reported (Barry et al., 2008).

McGowan and colleagues (2009) showed that exposure to early adversity was associated with epigenetic regulation of the GR receptor. In the area of attachment, epigenetic regulation of the serotonin transporter gene was found to influence the way in which adults cope with loss of attachment figures or other

trauma (Van IJzendoorn et al., 2010). The combination of GxE and epigenetics (Zhang & Meaney, 2010) seems to be the most promising avenue for investigating the complex interplay between genetic and environmental factors in explaining developmental outcomes, and in particular attachment security.