Infant attachment and stress regulation : a neurobiological study

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6 Discussion: Vulnerability and plasticity

In the largest cohort study of attachment to date, the Generation R study, with carefully assessed biological markers and behavioral observations, we were able to investigate parental and genetic influences on infant attachment and stress regulation. In the current series of studies, infant attachment quality was related to cortisol stress reactivity, as assessed before and after the SSP. Insecure-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, but disorganized infants displayed a more flattened slope than non-disorganized infants. Maternal lifetime depression appeared to be a risk factor that further elevated cortisol reactivity in infants with an insecure-resistant attachment relationship. Also, the genetic make-up of the child was associated with cortisol reactivity; carriers of the risk genotype of FKBP5, a gene involved in the negative feedback loop of the HPA-axis, showed higher levels of cortisol reactivity. Furthermore, an interaction between insecure-resistant attachment and FKBP5 was found, representing a double risk for heightened cortisol reactivity levels in infants who carry the FKBP5 risk genotype and at the same time have an insecure-resistant attachment relationship with their mother.

In our collaborative effort with the NICHD Study of Early Child Care and Youth Development (SECCYD) to identify potential attachment genes, we found no evidence for additive effects of candidate genes putatively involved in attachment security and disorganization. Furthermore, proposed risk models for DRD4, DRD2, and 5-HTT failed to provide unequivocal results. However, a co-dominant effect of the COMT Val/Met proved replicable across both studies. In carriers of the heterozygous Val/Met genotype, disorganization scores were higher compared to both Val/Val and Met/Met carriers. Investigating the additional effect of maternal care on attachment quality, we found that genetic variation in the mineralocorticoid receptor gene (MR), which is involved in HPA-axis functioning, modulated infants' sensitivity to care. Infants carrying the minor allele of MR 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 wildtype genotype of MR. The findings presented in this thesis provide attachment researchers with comprehensive results on vulnerability and plasticity factors in infant attachment and stress regulation. Moreover, the findings replicate and extend previous studies by making use of data from a large attachment cohort with physiological and genetic information.

Distribution of attachment in a large birth cohort
The assessment of attachment quality in a population based birth cohort provides the opportunity to compare the distribution of attachment classifications to meta-analytic findings. Outcomes from single well-powered studies are important, especially when heterogeneity plays a role in meta-analytic results. In the current study, the distribution of the attachment classifications was as follows: 58.6% secure (n = 486), 18.2% avoidant (n = 151), 22.4% resistant (n = 186). No classification could be assigned for n = 6 (0.7%) children. Of all children, 21.0% were classified as disorganized (n = 174), 79.0% were non-disorganized (n = 655). In Figure 1, the distribution of the current sample is presented together with the meta-analytic distribution of Van IJzendoorn et al. (1999), which represents a common distribution in non-clinical populations.

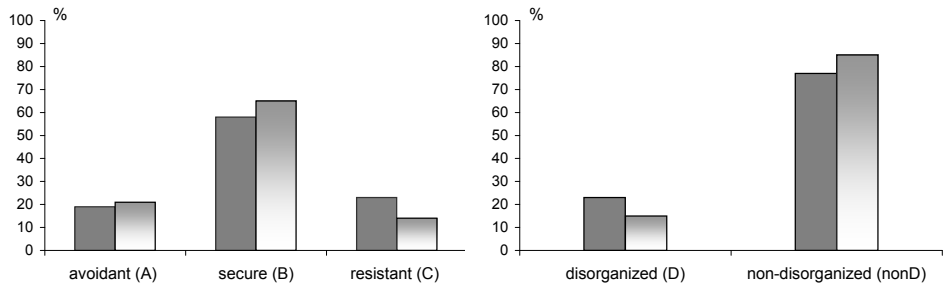

Figure 1. *Distribution of attachment classifications in the Generation R sample (solid bars) and from meta-analyses (shaded bars)*

In the current study, 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.

Physiological vulnerability in attachment
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. These findings document the vulnerability of insecure-resistant infants in physiological stress regulation, especially in combination with care from mothers with a lifetime diagnosis of depression. It could be argued that heightened stress reactivity in the insecure-resistant group should be interpreted as supporting an arousal model, assuming associations between behavioral and physiological activation during stress (Spangler & Schieche, 1998). To test whether increases in cortisol were related to the amount of crying (i.e. an index

of behavioral and physiological arousal) during the SSP, we used a measure of observed crying. When adding crying to the model, it was a significant covariate, but insecure-resistant attachment remained a significant predictor, indicating an effect of insecure-resistant attachment on cortisol reactivity *independent* of the amount of crying during the SSP.

We also showed that disorganized infants differed from non-disorganized infants in their diurnal cortisol rhythm, as they displayed a more flattened daily curve. The relation between attachment and infant diurnal rhythm of cortisol excretion has been largely neglected, and was for the first time explored in the current thesis. Our findings stress the disturbed nature of disorganized attachments as one of the most important risks for developmental psychopathology. Overall, the findings suggest differential physiological concomitants of avoidant, resistant, and disorganized attachments.

Genetic vulnerability in attachment

Quality of the parent-infant attachment relationship influences physiological stress regulation in infants (Bowlby, 1969/1982; Hertsgaard, Gunnar, Erickson & Nachmias, 1995). To extend the findings from previous studies, we added a genetic component, as genetic factors also contribute to the stress regulatory HPA-axis (Bartels et al., 2003; Steptoe et al., 2009; Wüst et al., 2004a). We found a significant interaction effect for insecure-resistant attachment and a variant in the FKBP5 gene, a co-chaperone of the glucocorticoid receptor gene involved in the negative feedback loop of the HPA-axis. This indicates a double risk for heightened cortisol reactivity levels in infants who carry risk alleles of the FKBP5 SNP *and* have an insecure-resistant attachment relationship with their mother. Resistant attachment and FKBP5 predispose infants to increased cortisol reactivity both independently as well as in interaction. These outcomes provide support for a double-risk model (Belsky et al., 2007) as the combination of environmental (indexed by resistant attachment) and genetic (FKBP5) risks increased stress reactivity in an additive way.

In an effort to identify potential 'attachment genes', we investigated polymorphisms in two cohorts; The Generation R study and the NICHD Study of Early Child Care and Youth Development (SECCYD). In both studies, no evidence emerged for additive effects of candidate genes putatively involved in attachment security and disorganization. Thus, genes in the dopamine, serotonin, oxytocin and neuroplasticity systems were not related to attachment quality. Previously reported associations for genes involved in attachment (DRD4 48 bp VNTR, 5-HTT) could not be replicated in the two cohorts. However, a co-dominant effect of COMT Val/Met proved replicable across studies. In carriers of the heterozygous Val/Met genotype, disorganization scores were higher compared to both Val/Val and Met/Met carriers. Co-dominant effects for COMT Val/Met have been reported for neurobehavioral functioning (Gosso et al., 2008; Wahlstrom et al., 2010) and

schizophrenia (for a meta-analysis, see (Costas et al., 2010). A greater range of gene expression in heterozygotes compared to homozygotes could play a role, providing a broader window for plasticity or response to stress (Comings & MacMurray, 2000). Evidence from this inquiry might suggest the latter, with COMT Val/Met carriers possibly being more susceptible to environmental influences, which in turn may increase risk for attachment disorganization. Moreover, COMT Val158Met has been shown to be involved in regulation of emotional arousal (Drabant et al., 2006), which is considered central to disorganized attachment. Disorganized infants' inability to regulate stress and emotions in arousing situations is striking, and their dysregulation has been documented as an early predictor of later psychopathology (Fearon et al., 2010; Sroufe et al., 2005). Findings from these studies support the idea of interplay between genetic and environmental factors in explaining developmental outcomes (Rutter et al., 2006), and provide evidence for environment-dependent genetic vulnerabilities in attachment and stress regulation.

Plasticity in attachment and stress regulation

Originally, GxE studies have focused mainly on double risk models (or: diathesis stress models; Rutter, 2006). Nevertheless, not all children are equally susceptible to risk factors, and studies on GxE interaction in attachment could benefit from a shift from a conventional model of vulnerability genes, or 'risk alleles', to a focus on plasticity or susceptibility (Belsky et al., 2009). From this perspective, certain genes are thought to render individuals more responsive than others to both positive *and* negative environmental experiences (Bakermans-Kranenburg & Van IJzendoorn, 2007; Belsky, Bakermans-Kranenburg & Van IJzendoorn, 2007). Applying the concept of differential susceptibility to the study of attachment, we found that infants carrying the minor allele of the mineralocorticoid receptor gene (MR) 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 wildtype 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 environment (maternal extreme insensitivity). As MR is involved in the fast onset of responses and associated with processing of stressful information (DeRijk & De Kloet, 2008), 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 (sensitive responsiveness) and negative parenting (extreme insensitivity), for better *and* for worse. This supports the differential susceptibility hypothesis (Belsky et al., 2007; Ellis, Boyce, Belsky, Bakermans-Kranenburg, & Van IJzendoorn, in press). When testing this hypothesis, careful assessment of the environment is essential. Defining the mere absence of adversity as a positive environment may lead to the under-detection

of differential susceptibility findings and an overrepresentation of vulnerability findings (Belsky et al., 2009). The use of observations of both negative and positive environmental factors makes it possible to accurately assess GxE processes in the present study.

Limitations

Some limitations of the current thesis 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 of effects, and not to an overestimation of the effects. Second, cortisol was sampled at 14 months of age, and 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. Fourth, 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. Fifth, maternal sensitive responsiveness and extreme insensitivity might be thought to reflect two extremes on a caregiving continuum. However, conceptually as well as statistically they indicate different, weakly related dimensions of parenting. Furthermore, 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). Sixth, we did not include maternal genotype in the present study, which could be associated with quality of maternal care (Bakermans-Kranenburg & Van IJzendoorn, 2008; Kaitz et al., 2010). This should be incorporated in future GxE investigations. When conducting GxE research, the environment and outcome should be assessed as carefully as the genotypes. Recently two meta-analyses have been published that failed to find a significant interaction effect between 5-HTTLPR genotype and stressful life events on depression (Munafo, Durrant, Lewis, & Flint, 2009; Risch et al., 2009). The authors of these meta-analyses conclude that the field had been too eager to accept GxE studies in the absence of genetic main effects, and that genome-wide association studies should be given priority (Risch et al., 2009). It should however be noted, as others have done (Bakermans-Kranenburg & Van IJzendoorn, 2010), that the selection of studies for inclusion in these meta-analyses was somewhat particular, and that the quality of the studies varied substantially, including

sometimes weak measures for life events (the environmental factor). In a narrative review on the same topic Uher and McGuffin (2008; 2010) reviewed all pertinent studies, showing that the method of assessment of environmental adversity was an important predictor of the outcome of the study. Detailed interview-based and observational approaches were associated with positive GxE findings, whereas all non-replications used self-report questionnaires. High-quality GxE studies with careful measurement of the environment and the outcome variables are needed, as well as explicit hypotheses about how a *specific* gene and a *specific* environmental condition interact to predict a specific outcome (Bakermans-Kranenburg & Van IJzendoorn, 2010). In the current study, we were able to apply these methods, providing robust results on GxE interplay in infant attachment and stress regulation.

Finally, genetic contributions to attachment may operate in ways not tested in here. For example, epistatic effects could play a role (e.g. Pezawas et al., 2008). Before evaluating these gene-gene interactions, more knowledge is needed about functionality and specific pathways of targeted genes. Also, effects of deletions or multiplications of larger DNA segments—copy number variations (CNVs)—are known to affect protein expression and gene function. These CNVs might act as vulnerability factors for neurodevelopmental phenotypes (Merikangas, Corvin & Gallagher, 2009). Furthermore, epigenetic processes merit consideration, as these can modify gene expression and neural function without changing nucleotide sequence (McGowan et al., 2009; Van IJzendoorn et al., 2010; Zhang & Meaney, 2010).

Clinical implications and future directions

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 low quality of maternal care, maternal depression or genetic risk. 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.

From a differential susceptibility view, our study shows that genetic make-up can modulate infants' openness to maternal care in both a negative and a positive way. A similar effect was found in a study of children with externalizing behavior problems (Bakermans-Kranenburg & Van IJzendoorn, 2006); children with the 7-repeat allele of the dopamine D4 receptor gene (DRD4) who were reared by insensitive mothers displayed more problem behaviors than children without the genetic variant. Carriers of the 7-repeat who were reared by sensitive mothers showed however the lowest levels of externalizing behavior. In the case of behavior problems, DRD4 seemed to moderate children's susceptibility to

parenting. The significance of viewing infants as susceptible instead of merely vulnerable provides major possibilities for intervention studies, and may help in explaining differential effectiveness of interventions. Recently, a moderating effect of the DRD4 gene was found on the effectiveness of an attachment based intervention (Video-feedback Intervention to promote Positive Parenting – Sensitive Discipline, VIPP-SD; Juffer, Bakermans-Kranenburg, & Van IJzendoorn, 2008). A larger intervention effect was found in children with the 7-repeat allele of the DRD4 gene (Bakermans-Kranenburg, Van IJzendoorn, Pijlman, Mesman, & Juffer, 2008). The plasticity of young children, for better or for worse, may provide behavioral scientists and clinicians with a framework that helps interpretation of seemingly confusing child developmental outcomes. Furthermore, future studies could benefit from incorporating both negative and positive environments in their designs, to fully capture the range of environmental influences in children's lives. As attachment is a complex behavioral phenotype in which polygenic effects might operate in combination with environmental factors, the most important effects might be hidden in gene-environment interactions. Promising avenues for future attachment studies are therefore the careful assessment of the interplay between (epi)genetic differences and child-rearing influences.