Infant attachment and stress regulation : a neurobiological study

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1 General introduction

Attachment and stress regulation: a study on vulnerability and plasticity

Vulnerability and plasticity are key concepts in infant development (Belsky, Hsieh, & Crnic, 1998; Rutter, 2006). Depending on the environment, vulnerabilities can predispose children to adverse outcomes. Plasticity on the other hand, is thought to induce both adverse *and* favorable outcomes, depending on the environment. For full comprehension of the attachment relationship formation, which is a developmental milestone in infancy, the identification of potential vulnerability and plasticity factors is essential, as insecure and disorganized attachments are major risk factors for later-life psychopathology (Fearon et al., 2010; Sroufe, Egeland, Carlson, & Collins, 2005). Parental and, more recently, neurobiological aspects have been associated with attachment quality (De Wolff & Van IJzendoorn, 1997; Fox & Hane, 2008), and also the interaction between these factors is of great interest to attachment researchers (Bakermans-Kranenburg & Van IJzendoorn, 2007). In the current thesis, vulnerability and plasticity in attachment and stress regulation are studied in the largest population based attachment cohort to date.

Importance of quality of care

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 & Meaney, 1993). In the first year of life, regulation and coping are primarily externally organized, which 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 is expected to lead to differences in attachment quality in the infant (Sroufe, 1997). Maternal sensitive responsiveness to the baby's 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 and, potentially, psychopathology elevate the risk for insecure attachment (Cummings & Davies, 1994; Out, Bakermans-Kranenburg & Van IJzendoorn, 2009).

The case of depression. Maternal depression has 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 significant associations 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). Depression may also negatively influence infants' physiological regulation. More specifically, in several studies maternal depression was related to higher levels of stress hormones 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).

Attachment and stress regulation: cortisol

Hertsgaard, Gunnar, Erickson, and Nachmias (1995) suggested that assessment of cortisol levels may be particularly useful in attachment research. The neuroendocrine system is stimulated when coping behaviors are inadequate or coping sources are unavailable, which are crucial aspects of unresponsive maternal care and subsequent insecure attachment relationships. 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).

Various studies have tested the effect of stressful events on HPA-axis functioning in infants, most of them focusing on cortisol levels around the stressful Strange Situation Procedure (SSP; Ainsworth, Blehar, Waters & Wall, 1978) 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 disorganized infants (Hertsgaard et al., 1995; Spangler & Grossmann, 1993; Spangler & Schieche, 1998), whereas 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).

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, variation in cortisol reactivity for the different attachment categories may be related to systematic differences in diurnal rhythms.

Genetics of stress regulation

Associations between attachment quality and cortisol levels implicate that cortisol levels are, at least partly, determined by the caregiving environment (Meaney, 2001; Gunnar & Quevedo, 2007). Genetic factors have not received much attention, although there is ample evidence that these play a role in explaining variance in HPA-axis activity (Bartels et al., 2003; Steptoe et al., 2009; Wüst et al., 2004a). Recently, specific candidate genes involved in explaining variability in cortisol levels have been identified. Several studies focused on the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR), which mediate many of the effects of mineralocorticoids and glucocorticoids, respectively. Within GR, several molecules, so-called chaperones and co-chaperones, play a critical role. An important co-chaperone is the FKBP5 gene. Genetic variants of the GR, MR and FKBP5 gene (single nucleotide polymorphisms; SNPs), appear to contribute to interindividual variability in HPA-axis and are crucial in the onset and recovery from stress. This in turn is essential for healthy physiological and behavioral regulation (Binder, 2009; Ising et al., 2008; Kumsta et al., 2007; Wüst et al., 2004b). As the infant-parent attachment relationship can be considered the infant's most important emotion regulation system (Bowlby, 1969/1982, Cassidy, 1994), the role of a genetic factor influencing homeostasis might be of great importance.

Genetics of attachment

Next to a genetic factor involved in stress regulation, the 'usual suspects' (Ebstein, 2010), genetic variants in the dopaminergic, serotonergic, oxytonergic, and neuronal plasticity systems, may play a role in the quality of infants' attachment behavior. The dopaminergic system is involved in attentional, motivational, and reward mechanisms (Robbins & Everitt, 1999). Common variations in dopaminergic genes DRD4 48 bp VNTR, DRD4 -521C/T, DRD2/ANKK1 and

COMT Val158Met are associated with regulation of dopamine levels (D'Souza & Craig, 2006). Carrying the minor allele of these polymorphisms (respectively, DRD4 48 bp 7-repeat; DRD4 -521 C; DRD2/ANKK1 T[A1]) has been related to variations in infant temperament (Ebstein, 2006) and ADHD (Faraone & Khan, 2006). A protective effect has been reported for COMT heterozygotes (Val/Met) showing dopamine levels associated with optimal neurobehavioral outcomes, compared with both homozygous groups (Wahlstrom, White & Luciana, 2010). The serotonin system is involved in affect and emotion. The short variant of the serotonin transporter gene 5-HTT (5-HTTLPR) is associated with less efficient transcription and serotonin uptake in the synapse (Greenberg et al., 1999; Heils et al., 1996), and is related to psychiatric disorders (Ebstein, 2006; Rutter, 2006). The oxytonergic system is related to social and parenting behaviors, and both oxytocin levels and variants in the oxytocin receptor gene (OXTR rs53576 and rs2254298; in particular the minor A-allele) are associated with the formation of social bonds in both human and animal studies (Bakermans-Kranenburg & Van IJzendoorn, 2008; Carter et al., 2009; Feldman et al., 2010, Insel, 2010). Finally, brain-derived neurotrophic factor (BDNF) is a protein associated with neuronal growth and survival (Gizer, Ficks & Waldman, 2009). The gene coding for this protein, also called BDNF, contains a polymorphism influencing secretion of BDNF in the brain. This polymorphism (especially the minor Met-allele) is associated with ADHD (Gizer, Ficks & Waldman, 2009) and responses to stress and adversity; children with the Met-allele exposed to early deprivation manifest increased anxiety (Casey et al., 2009). Several studies have been undertaken to identify potential attachment genes (Bakermans-Kranenburg & Van IJzendoorn, 2004; 2007; Lakatos et al., 2000; Spangler, Johann, Ronai & Zimmerman, 2009), providing confusing results which call for replication in a large, ethnically homogeneous sample (Burmeister, McInnis & Zollner, 2008).

Gene-environment interaction

Both environmental factors and genes may affect the attachment relationship and infant stress regulation. The most important effects on child development are probably hidden in interactions between genetic and environmental factors (Barry, Kochanska & Philibert, 2008; Belsky et al., 2009). Gene-environment interactions can take various forms. One is a double risk model (or diathesis stress model; Rutter, 2006), in which some individuals are at heightened risk – because of their genetic make-up – for negative outcomes in the face of adversity, whereas persons without the genetic vulnerability are less affected by adversity (e.g. Caspi et al, 2002). Another specific type of gene-environment interaction is known as differential susceptibility (Belsky, Bakermans-Kranenburg & Van IJzendoorn, 2007; Belsky et al., 2009), where certain genes are thought to render individuals more responsive than others to both positive *and* negative environmental experience. In other words: 'the very same individuals who may be most adversely

affected by many kinds of stressors, may simultaneously reap the most benefit from environmental support and enrichment' (Belsky et al., 2007). In this model, individuals are thought to vary in their plasticity to environmental influences, and studies on GxE interaction in attachment may benefit from a shift from a conventional model of vulnerability genes, or 'risk alleles', to a focus on plasticity or susceptibility.

Attachment in Generation R

The influences of environmental and genetic factors on attachment and stress regulation were studied in the Generation R study. The Generation R study was designed to identify early environmental and genetic determinants of growth, development and health from fetal life into young adulthood in Rotterdam, the Netherlands (Jaddoe et al., 2007; 2008). Detailed measurements of the child's development were obtained 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 (see Figure 1). The Generation R study provides ample information for investigating research questions on environmental and biological factors involved in attachment and stress regulation, and is the largest study with data on attachment, observed parenting and biological markers to date.

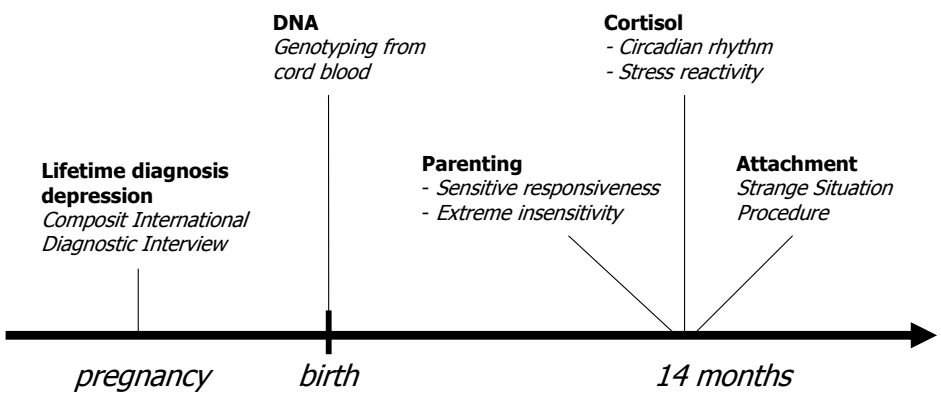

Figure 1. *Assessments in Generation R used in current thesis*

Aims of the study

The general aim of the current thesis is to provide more insight into the role of parental and biological factors in the development of the infant-mother attachment relationship. Both observational and experimental measures were used to assess

these associations, including observed behavior, physiological and genetic markers, and interviews. The main focus of Chapter 2 is the association between quality of attachment and cortisol levels, both cortisol stress reactivity and cortisol circadian rhythm. Moreover, the moderating effect of maternal depression on this association is explored. Chapter 3 extends the current knowledge on cortisol and attachment by adding a genetic component. In Chapter 4 we examine the interaction between genes and early caregiving environment on attachment security. Chapter 5 gives an overview of the molecular genetics of attachment, presenting the findings of an investigation in collaboration with the NICHD Study of Early Child Care and Youth Development (SECCYD). The effects of candidate genes on attachment quality are tested in a large-scale combination of two birth cohorts, providing a unique possibility for immediate replication.