Differential susceptibility to parenting: Exploring new approaches

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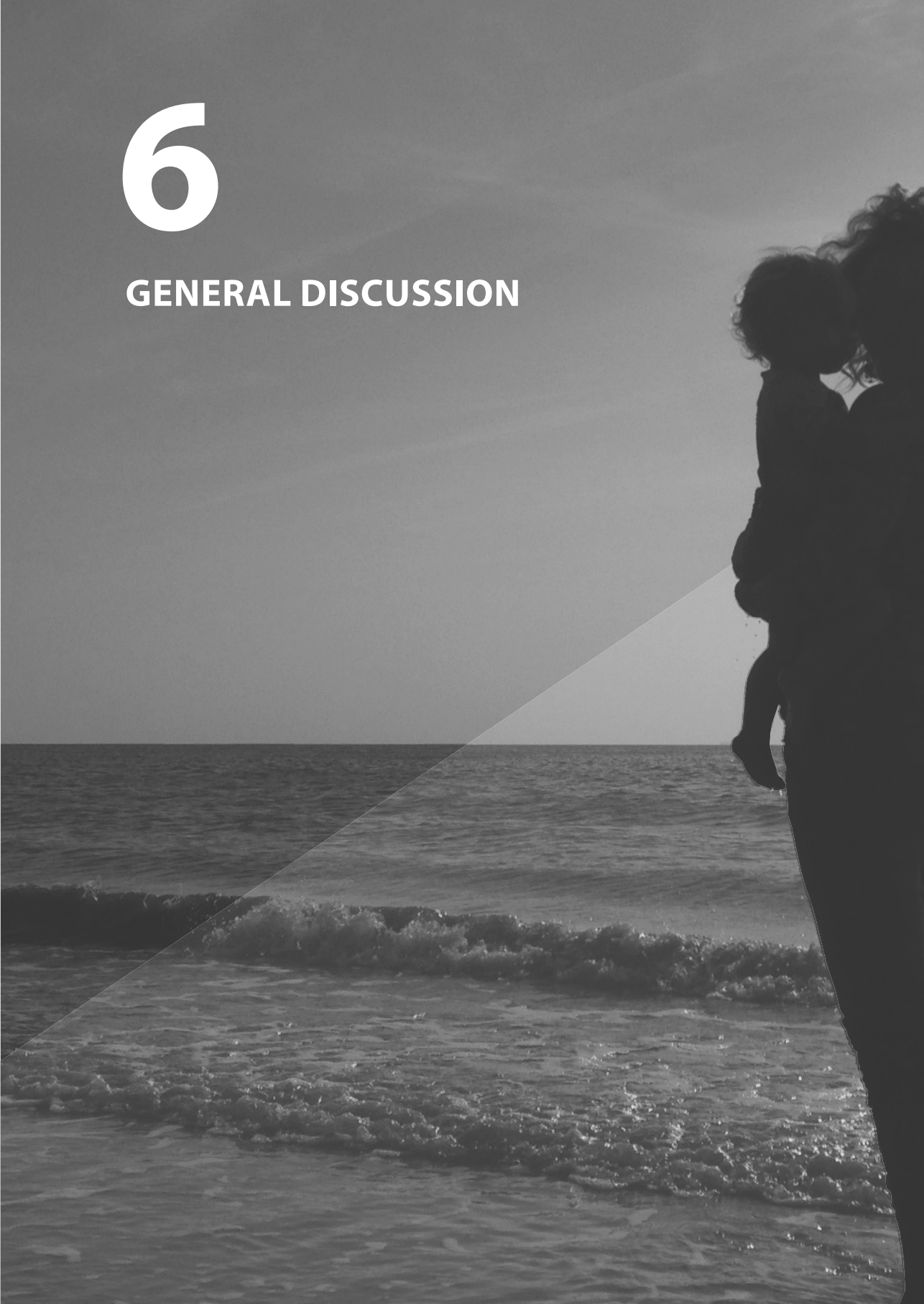
GENERAL DISCUSSION



INVESTIGATING DIFFERENTIAL SUSCEPTIBILITY TO PARENTING: BEYOND COMMON METHODS

Over the last decades, there has been increased interest in the interplay of biological and environmental factors predicting child development. One observation that has emerged is that not all children seem equally susceptible to the effects of parenting. Such differences in susceptibility are thought to be due to genetic, temperamental, or physiological susceptibility factors. Recent research has explored a broad variety of susceptibility factors, environmental factors, and outcomes. In this thesis, we conducted a series of studies that offer important extensions to current methods in differential susceptibility research. First, we investigated differential susceptibility from a developmental perspective by including multiple measures over time (Chapter 2). Second, we went beyond single-gene/polymorphisms in the investigation of gene-environment interplay by aggregating genetic variation in a set of dopamine genes (Chapter 3). Third, we extended previous research on mild perinatal adversity as a prenatal susceptibility factor, by examining its moderating role in the association between harsh parenting and hair cortisol levels (Chapter 5), after careful assessment of background factors that should be taken into account when hair cortisol levels are investigated (Chapter 4).

TESTING DIFFERENTIAL SUSCEPTIBILITY OVER TIME

In Chapter 2, we examined the longitudinal effects of gene-environment interplay between maternal sensitivity and DRD4 genotype on externalizing behavior across the first five years of life, in an ethnically homogenous subsample of the Generation R Study. Previous research showed that DRD4 genotype moderates the association between maternal sensitivity and externalizing behavior in children (e.g. Bakermans-Kranenburg & Van IJzendoorn, 2006). However, in previous studies only single time points for the assessment of maternal sensitivity and externalizing behaviors had been included. In contrast, we included multiple measures of both maternal sensitivity (14, 36 and 48 months) and externalizing behavior (18 months, 36 months, 5 years). In this study, we focused only on externalizing– not internalizing- behavior as the outcome measure, for two reasons. First, this study was a replication and extension of a previous study, that reported an interaction effect of DRD4 genotype and maternal sensitivity for externalizing behavior (Bakermans-Kranenburg & Van IJzendoorn, 2006). Second, internalizing behavior is more difficult to assess at a young age, since it pertains to an internal state in contrast to overt externalizing behaviors. Internalizing behavior is less prevalent at a young age and internal consistencies of measures are lower compared to externalizing behavior (e.g. Gilliom & Shaw, 2004). Significant interaction effects of maternal sensitivity at 14 months and DRD4 genotype on externalizing behavior were found at 18 and 36 months. At 18 months, the results were consistent with the differential susceptibility model. Children with at least one DRD4 7-repeat showed the lowest levels of externalizing behavior in cases of high maternal sensitivity, but the highest levels when the mother was insensitive, compared to children without a 7-repeat allele. Externalizing behavior at age 5 was predicted by insensitive parenting at 48 months, independent of DRD4 genotype. One of the

notable strengths of this study was that a structural equation model including all measures across time supported the differential susceptibility model: The overall effect of early maternal sensitivity on later externalizing behavior was significant only for children with a DRD4 7-repeat allele. Thus, DRD4 genotype moderated the effects of early, but not later maternal sensitivity on child externalizing behavior.

FROM SINGLE GENETIC MARKERS TO A GENE-SET APPROACH

In Chapter 3, we extended current research of gene-environment interplay by examining how harsh parenting at age 3 interacts with genetic variation across multiple child dopamine genes in shaping child externalizing behavior assessed at age 5. Most studies in this area conventionally employ single candidate genes, often related to the dopamine system, a neurotransmitter system involved in attentional, motivational and reward mechanisms (Robbins & Everitt, 1999). Several dopaminergic polymorphisms have shown to moderate the association between parenting and child behavior (Bakermans-Kranenburg & Van IJzendoorn, 2011). However, complex traits such as problem behavior are suggested to be polygenic (Reif & Lesch, 2003). Thus, single candidate genes might be limited in explanatory power. A different approach – hypothesis-free genome-wide testing of gene-environment interaction effects – tests individual SNPs independently on a one-by-one basis. Due to the enormous sample size required to detect small individual effects with this approach, a combination of approaches might be best. In this study, we aggregated genetic variance of multiple SNPs within a *set* of dopamine genes and we tested the joint effect of these SNPs. This improves statistical power by reducing multiple testing, and yet includes multiple theoretically (biologically) related genetic markers. We tested the association between variation in the dopamine-gene set and externalizing behavior, in a sample stratified for harsh parenting. Furthermore, we examined the effects of paternal, maternal, and pooled harsh parenting separately. The association between variance in the dopamine gene-set and externalizing behavior was statistically significant or approached significance for children without harsh parenting experiences, but was absent in the group with harsh parenting. Our results are suggestive of gene-environment interplay. Specifically, variance in a dopamine gene-set appeared to predict externalizing behavior in a low-risk environment (no harsh parenting). However, in a higher-risk environment (harsh parenting) the genetic effects might be overruled by environmental influences. A disadvantage of this approach is that the JAG tool requires a dichotomous rather than continuous environmental measure, and that it is not possible to infer the direction of effects between genotypes and externalizing behavior (i.e. which genetic variants confer greater or lesser risk), because it yields a *combined* statistical effect. Therefore, it is not possible yet to test differential susceptibility this way. However, our results show that the gene-set approach is a promising alternative or complement to candidate gene and GWEI studies in understanding the role of genetic pathways in gene-environment interplay. The approach is still developing and it is expected to be of valuable use in differential susceptibility research in the future.

MILD PERINATAL ADVERSITY AS SUSCEPTIBILITY FACTOR

Hair cortisol has been validated as a biomarker of chronic stress in both adults (e.g., Manenschijn, Koper, Lamberts, & Van Rossum, 2011; Stalder & Kirschbaum, 2012) and children (Noppe et al., 2014; Vanaelst et al., 2012). Since this is a relatively new measure of stress, we first examined which background variables should be taken into account when studying hair cortisol and cortisone in Chapter 4. Our results indicated that studies on hair cortisol and cortisone levels should at least take into account the potentially confounding role of gender, BMI, ethnicity, SES variables such as family income and the number of persons living of this income, and hair characteristics such as hair color and time since last wash. In Chapter 5, we investigated the influence of maternal harsh parenting at age 3 on 6-year old children's hair cortisol levels. We also explored whether this association is moderated by mild perinatal adversity (late prematurity or low birth weight at full term birth). Mild perinatal adversities are associated with physiological adaptions, including increased stress reactivity (Economides, Nicolaides, Linton, Perry, & Chard,1988; Jones et al., 2006; Wüst, Entringer, Federenko, Schlotz, & Hellhammer, 2005). Previous research has shown that children with a history of mild perinatal adversities are more susceptible to environmental influences than children without perinatal adversities, for better and for worse (Van der Kooy-Hofland, Van der Kooy, Bus, Van IJzendoorn, & Bonsel, 2012). We showed that mild perinatal adversity moderated the association between maternal harsh parenting and children's hair cortisol levels. There was a negative association between maternal harsh parenting and cortisol levels, but only for children with mild perinatal adversity. These children showed the *lowest* cortisol levels in case of higher levels of maternal harsh parenting, consistent with a down-regulation of the HPA-axis as a result of chronic stress (Fries, Hesse, Hellhammer, & Hellhammer, 2005; Gunnar & Vazquez, 2001). On the other hand, children with mild perinatal adversity showed the *highest* cortisol levels in the absence of harsh parenting. These higher cortisol levels could reflect heightened basal cortisol levels or higher stress reactivity to the environment as a correlate of mild perinatal adversity (Economides et al., 1988; Phillips et al., 1998, 2000). An overall greater reactivity to the environment could be beneficial in positive environments (Boyce & Ellis, 2005). Our results again suggest mild perinatal adversity is a susceptibility marker, increasing susceptibility to both positive and negative environments.

DEVELOPMENT OF DIFFERENTIAL SUSCEPTIBILITY

Developmental considerations

The results described in Chapter 2 show the importance of investigating differential susceptibility over time, rather than focusing on single time points. If we had focused on the last time point only, we would have missed the early GXE effect, while if we only had focused on the early time point, we might have wrongly concluded that there was a stable gene-environment interaction. The absence or presence of an interaction effect at a certain point in time does not mean that this interaction effect is stable. The exact timing of plasticity differences might differ across populations and ages. There might be different sensitivity periods, which may differ across susceptibility markers, phenotypes, and environmental

factors. The mechanisms underlying these fluctuations in plasticity remain to be further investigated. It is important to note that the developmental aspect of differential susceptibility indicates that the selection of age-ranges has consequences for the (non)replication of previous findings.

Factors influencing plasticity

Our results emphasize the plasticity of differential susceptibility. It is not a static 'trait', but a dynamic feature that is influenced both by nature *and* nurture (Pluess & Belsky, 2011). Susceptibility factors like physiological stress reactivity and temperament are partially influenced by environmental experiences, both prenatal and postnatal. Prenatal stress might promote (postnatal) developmental plasticity, thereby enhancing adaptation to the postnatal environment through the influence on physiological and behavioral markers of susceptibility (Pluess & Belsky, 2011) and through prenatal epigenetic changes (e.g. Oberlander et al., 2008). These associations may however be moderated by child genotype (Pluess et al., 2011). The results described in Chapter 5 are consistent with prenatal programming of postnatal plasticity, as children with mild perinatal adversity show an enhanced susceptibility to environmental influences. Similarly, postnatal environmental experiences can also influence plasticity. So, the plasticity of susceptibility can be viewed as a phenotype on its own. It is likely to be regulated through many different pathways (Pluess & Belsky, 2011), through direct and indirect genetic contributions, prenatal and postnatal environmental effects, and interaction effects of genetic and/or environmental influences. Additionally, environmental factors can influence gene-expression through epigenetic changes that could influence the functionality of genotype. This complex dynamic nature is important to take into account when investigating differential susceptibility.

INVESTIGATING DIFFERENTIAL SUSCEPTIBILITY IN THE GENERATION R STUDY

This study is embedded in the Generation R Study, a large prospective population-based cohort. The longitudinal design resulting in a large data set containing a broad scale of variables from prenatal life onwards (Jaddoe et al., 2012) provides many possibilities to investigate differential susceptibility in a large sample. The sample is relatively homogenous, especially for the genetic studies in which only Caucasians were included. Ethnic homogeneity is an advantage when studying gene X parenting interplay, since there are differences across ethnic backgrounds in both parental behavior (e.g. Deater-Deckard et al., 2011) and gene function (e.g. Williams et al., 2003, but see Vijayendran et al., 2012). However, homogeneity can be a disadvantage with regard to environmental factors and phenotypes. The distributions of many variables are skewed; for instance, the majority of the Generation R sample is of relatively high SES. The homogeneity of the sample may be increased over time by non-random attrition. Homogeneity of the study sample makes it difficult to generalize our findings to other, more diverse populations, and may provide an underestimation of the effect sizes. Other limitations also need to be discussed. First of all, the sample sizes we used are still considered small for genetic studies. Large GxE effect sizes require sample sizes of at least 600 participants to reach sufficient statistical

power (80%) (Duncan & Keller, 2011). Second, a drawback of such a large sample is that the careful assessment of environment and outcome measures is difficult due to practical reasons. Many measures are assessed through questionnaires. For example, we assessed harsh parenting through self-report. Due to social desirability, self-report may result in the underreporting of harsh parenting behavior. Self-reported parenting is thought to reflect parental attitudes more than the actual parenting behavior toward the child (Hoff, Laursen, & Tardif, 2002). Furthermore, self-report might be influenced by characteristics of the parents and their ability to reflect on their own behavior (Aspland & Gardner, 2003; Hoff et al., 2002). In the studies described in this thesis, we also relied on parent-report of child externalizing behavior because of availability of the data and the repeated assessment in our sample. Correlations between different reporters of child problem behavior are moderate (Van der Ende, Verhulst, & Tiemeier, 2012). Information from multiple reporters (such as child self-report or teacher-report) should be included in addition or in combination, whenever feasible. In our studies, we tried to use objective measures whenever possible, for example observational measures of maternal sensitivity (Chapter 2) and hair cortisol as a measure of chronic stress (Chapter 5). Furthermore, we tried to prevent common method bias by combining measures from different sources. This was often possible, except in the study presented in Chapter 3 when we used parent-report of parenting behavior as well as child externalizing behavior, as these measures were available in the largest available sample. This was necessary because of sample requirements for the gene-set analyses. In future studies, the use of more objective measures is however recommended. Finally, in Chapters 4 and 5 we use hair cortisol, a relatively new biomarker of chronic stress. It is a promising measure, reflecting a new dimension of HPA axis activity, and it is shown to be associated with stress in a variety of situations (Staufenbiel, Penninx, Spijker, Elzinga, & Van Rossum, 2013). However, there are still many aspects of hair cortisol that remain to be investigated, such as genetic influences on hair cortisol and how hair cortisol concentrations relate to short-term stress.

RECOMMENDATIONS FOR FUTURE RESEARCH

Over the last years, the investigation of differential susceptibility has expanded enormously, with a large variety of environmental factors, outcomes, susceptibility factors, in different populations and age ranges (Bakermans-Kranenburg & Van IJzendoorn, 2015). With the growing interest in differential susceptibility, the methods that are used to investigate differential susceptibility are also developing. For instance, the evaluation of differential susceptibility versus other interaction models has shifted from simple eyeballing to more stringent statistical tests (Belsky, Pluess, & Widaman, 2013; Roisman et al., 2012; Widaman et al., 2012). The studies described in this thesis also show extensions of current approaches by looking at differential susceptibility from a developmental perspective, aggregating genetic variance across multiple polymorphisms within a gene-set, and investigating mild perinatal adversity as a susceptibility factor in a different context.

Future studies investigating genetic differential susceptibility could look beyond the 'usual suspects' and include other genotypes as potential susceptibility markers. Second, future studies

should try to shed some more light on the underlying mechanisms of differential susceptibility: what are determining factors and how is susceptibility influenced over time? The interplay of the different factors can be examined by integrating studies of susceptibility markers that before were investigated in an independent manner. It might be especially important to include measures of DNA methylation as gene expression affects the functional significance of genotype (McGowan & Roth, 2015; Van IJzendoorn, Bakermans-Kranenburg, & Ebstein, 2011). Furthermore, many studies on differential susceptibility are merely correlational. Experimental studies are needed to further our understanding of the underlying mechanisms and to investigate causal relationships. Advantages of experimental studies compared to correlational studies include enhanced power and reduced measurement error. Furthermore, random assignment of participants to groups while stratifying for the susceptibility markers can ensure independence of the environmental factor and the susceptibility factor (Bakermans-Kranenburg & Van IJzendoorn, 2015). Another recommendation refers to the importance of careful assessments of environmental and outcome measures. For instance, it is shown to be essential for GxE results that E is assessed in a proper way (Uher & McGuffin, 2008, 2010). Finally, as always, replication studies are important to rule out chance findings.

To conclude, when investigating differential susceptibility it is important to be aware of the dynamic nature of susceptibility: the complex interplay of different susceptibility markers and the developmental changes. It is unlikely that a single, stable, definitive susceptibility marker will be found. Examining differential susceptibility has important implications, for example with regard to interventions. The -often- modest effect estimates of interventions in different domains are likely to be underestimated, because individuals may differ in their susceptibility to the intervention (Bakermans-Kranenburg & Van IJzendoorn, 2015; Belsky & Van IJzendoorn, 2015). Understanding what works for whom and why, might enable a more targeted approach.

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