Differential susceptibility to parenting: Exploring new approaches

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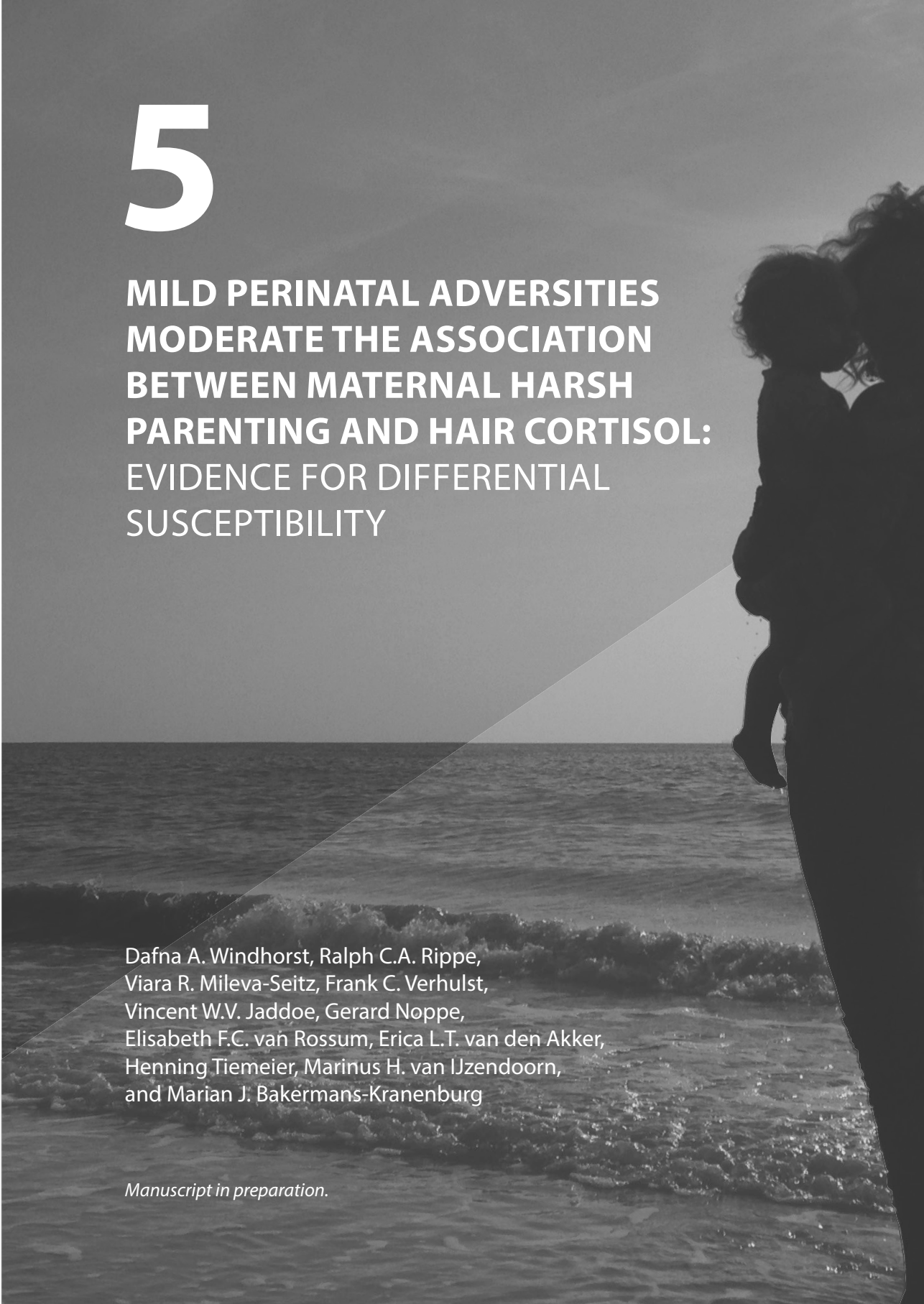
MILD PERINATAL ADVERSITIES MODERATE THE ASSOCIATION BETWEEN MATERNAL HARSH PARENTING AND HAIR CORTISOL: EVIDENCE FOR DIFFERENTIAL SUSCEPTIBILITY



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ABSTRACT

Children with mild perinatal adversities were previously shown to have an enhanced susceptibility to environmental influences, for better and for worse. In a large population-based cohort study ($N = 1,776$), we investigated whether mild perinatal adversity moderated the association between maternal harsh parenting and hair cortisol levels, a biomarker of chronic stress. Mild perinatal adversity was defined as late preterm birth (gestational age at birth of 34-37 weeks, 6 days) or small for gestational age (birth weight between the 2.5th and 10th percentile for full term gestational age). Harsh parenting was assessed by maternal self-report at 3 years. Hair cortisol concentrations were measured from hair samples collected at age 6. Mild perinatal adversities moderated the association between maternal harsh parenting and hair cortisol levels. Children with mild perinatal adversity had lower cortisol levels if parented more harshly and higher cortisol levels in the absence of harsh parenting than children who did not experience mild perinatal adversity. These results provide further evidence that mild perinatal adversities increase differential susceptibility to environmental influences.

Key words: mild perinatal adversities, differential susceptibility, hair cortisol, HPA axis, parenting

INTRODUCTION

Hypothalamus-pituitary adrenal (HPA) axis activity in young children is partially regulated by parental care (Adam, Klimes-Dougan, & Gunnar, 2007; Gunnar & Donzella, 2002). However, children differ in their susceptibility to the effects of parental care and other environmental influences due to genetic, behavioral, or physiological susceptibility factors (Ellis, Boyce, Belsky, Bakermans-Kranenburg, & Van IJzendoorn, 2011). Previous research has shown that children with a history of mild perinatal adversities (late prematurity or low birth weight at full term birth) are more susceptible to environmental influences than children without perinatal adversities (Van der Kooy-Hofland, Van der Kooy, Bus, Van IJzendoorn, & Bonsel, 2012). The aim of the current study is to investigate the influence of maternal harsh parenting on 6-year old children's hair cortisol levels, a biomarker of chronic stress, and to explore whether this association is moderated by mild perinatal adversity.

The glucocorticoid hormone cortisol is the end product of the HPA axis, one of the major activation or stress systems of the body. In response to physical and psychosocial stressors, activation of the HPA axis results in a short-term increase of cortisol levels. Cortisol serves different functions, including increased energy for action during stress and the activation of other stress systems (De Kloet, Joëls, & Holsboer, 2005; Sapolsky, Romero, & Munck, 2000). Apart from stress-induced peaks in cortisol, basal cortisol levels follow a diurnal rhythm characterized by a post-waking peak in the morning followed by a decline during the rest of the day (Kirschbaum & Hellhammer, 1989). Chronic exposure to stress may result in adaptations of HPA axis activity, including altered stress reactivity and dysregulation of diurnal rhythms (Miller, Chen, & Zhou, 2007). While stress is initially linked to increased HPA axis activity accompanied by higher cortisol levels, lower than normal cortisol levels are also reported as a consequence of stress. Such hypocortisolism may be the result of down-regulation of the HPA axis due to chronically elevated cortisol levels (Gunnar & Vazquez, 2001; Hostinar, Sullivan, & Gunnar, 2014). Moreover, associations between HPA axis activity and child behavior may be moderated by age. Meta-analytical results showed that externalizing behavior is associated with *higher* basal cortisol levels (hyperactivity) in pre-schoolers, but with *lower* basal cortisol levels (hypoactivity) in elementary school-aged children (Alink et al., 2008).

Hair cortisol is a relatively new biomarker of chronic stress. It has quickly become a popular choice in stress studies (Russell, Koren, Rieder, & Van Uum, 2012; Stalder & Kirschbaum, 2012), because of its advantages compared to assessments of cortisol through saliva, urine, or blood. The relatively stable growth rate of hair (1 cm per month) enables the retrospective and stable assessment of cortisol levels and cortisol production over time (Russell et al., 2012), where traditional methods enable collecting data about momentary cortisol levels and are more sensitive to other factors such as time of day, sleep, and food (Adam et al., 2007). Furthermore, sampling is easy and non-invasive, samples can be transported and stored easily, and the measurement methods are relatively simple (Noppe et al., 2014; Rippe et al., in prep). 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).

Experimental animal studies have shown that parental care is an important regulator of HPA axis activity in the early lives of rodents and nonhuman primates (e.g., Hostinar et al., 2014; Levine, 2001). In humans, HPA axis activity is also known to be regulated by the social environment, and during childhood in particular by parental care (Gunnar & Donzella, 2002; Gunnar & Quevedo, 2007; Hostinar et al., 2014). Parental caregiving can buffer cortisol increases in children in response to external stressors. It has been suggested that sensitive and responsive care allows children to better cope with stress without increases in cortisol levels (Adam et al., 2007; Gunnar & Quevedo, 2007), whereas insensitive caregiving is associated with increased HPA axis reactivity (Gunnar & Quevedo, 2007; Hastings et al., 2011). Associations between parental care and basal cortisol levels have also been found (e.g., Pendry & Adam, 2007). In cases of extremely adverse care, such as child abuse and neglect, both disrupted cortisol reactivity to stressors as well as elevated and blunted diurnal rhythms are reported (Tarullo & Gunnar, 2006).

In the current study, we investigated the effects of harsh parenting at age 3 on hair cortisol levels at age 6 in a large population-based cohort. Harsh parenting behavior includes negative emotional interaction and coercive acts of the parent directed to the child, such as name calling, yelling, and threatening (Chang, Schwartz, Dodge, & McBride-Chang, 2003). The prevalence of such parenting practices is substantial (McLoyd & Smith, 2002; Straus & Field, 2003), as was also shown in a study using data from the present cohort (Jansen et al., 2012). Harsh parenting contributes to a variety of negative outcomes including child emotional and behavioral problems, poor self-esteem, and low school achievement (Larzelere, 2000; Mackenbach et al., 2014; Solomon & Serres, 1999; Teicher, Samson, Polcari, & McGreenery, 2006).

The differential susceptibility hypothesis proposes that some children, who according to the diathesis-stress framework (Zuckerman, 1999) and the dual-risk model (Sameroff, 1983) are more vulnerable to adverse environments, may in fact be more susceptible to both negative *and* positive effects of the environment due to a variety of susceptibility markers including genetic, temperamental, and physiological factors (Belsky, Bakermans-Kranenburg, & Van IJzendoorn, 2007; Ellis et al., 2011). Physiological susceptibility to context was introduced in a study by Boyce et al. (1995). Highly biologically reactive children, who had increased cardiovascular or immune reactivity to stressors, showed the highest respiratory illness incidences in high-adversity childcare or home environments, but the lowest illness rates in supportive environments compared to children with low biological reactivity (Boyce et al., 1995).

These findings led to the reconceptualization of physiological reactivity that was transformed into biological sensitivity to context with less emphasis on its negative effects (Boyce & Ellis, 2005). A heightened biological sensitivity may be disadvantageous in adverse environments, but beneficial in supporting environments (Boyce & Ellis, 2005; Ellis et al., 2011; Obradović, 2012). Mild perinatal adversities are associated with physiological adaptations, including increased stress reactivity (Economides, Nicolaides, Linton, Perry, & Chard, 1988; Jones et al., 2006, Wüst, Entringer, Federenko, Schlotz, & Hellhammer, 2005). In light of the biological sensitivity-to-context theory, mild perinatal adversity would make children more sensitive to environmental influences. Indeed, in a randomized control trial, 5-year-old children with a history of mild perinatal adversities (defined as late preterm

birth or small for gestational age but full term) showed the lowest literacy levels in a control group, but outperformed children without such adversities following a web-based remedial literacy intervention, both immediately after the intervention and 8 months later. In fact, the effect of the intervention was absent in children without mild perinatal adversities (Van der Kooy-Hofland et al., 2012). These results indicate that mild perinatal adversities make children more susceptible to environmental influences, for better and for worse.

In the current study we examined the effects of harsh parenting at child age 3 on hair cortisol levels at age 6. Our hypothesis was that children with a history of mild perinatal adversity, defined by late prematurity or low birth weight at full term birth (as in Van der Kooy-Hofland et al., 2012), would be more susceptible to the effects of harsh parenting and show dysregulated (either increased or decreased) cortisol levels with increasing levels of maternal harsh parenting. We expected that this association would be absent for children without a history of perinatal adversity.

METHOD

Setting

The current study used a subsample of children participating in the Generation R Study, a prospective cohort investigating development from fetal life into young adulthood in Rotterdam, the Netherlands (Jaddoe et al., 2012, Tiemeier et al., 2012). Briefly, all pregnant women living in Rotterdam with an expected delivery date between April 2002 and January 2006 were invited to participate. The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all adult participants.

Study population

The sample for the current study consisted of 1,776 children, for whom measures of maternal harsh parenting and hair cortisol were available. Hair samples for cortisol assessment were collected during a lab visit at age 6. However, hair collection did not immediately start at onset of this research wave. Of the 6,690 children that visited the Generation R research center during this wave, the last 3,570 children were asked to participate in hair sample collection, and 3,034 children responded positively (85%). Hair cortisol concentration was successfully quantified in 2,984 children. Participants were excluded in case of systemic glucocorticoid use (n = 8). For the remaining 2,976 children, maternal harsh parenting data were available for 1,893 children, for which gestational age and birth weight were known for 1,888 children. Twins (n = 39) were excluded, because these children tend to have lower birth weights (Poulter, Chang, MacGregor, Snieder, & Spector, 1999). If mothers had participated with multiple singleton children, one child per sibling pair was randomly excluded from the analyses (n = 25). Finally, 48 children were excluded because of extreme rather than mild perinatal adversity, i.e. gestational age below 34 weeks or birth weight below the 2.5th percentile in case of full term birth. The final sample for the current study thus consisted of 1,776 children. Sample characteristics are described in Table 1.

Table 1 | Sample characteristics

	Total sample	**Group without mild perinatal adversity**	**Group with mild perinatal adversity**	
	***n* = 1776**	***n* = 1515**	***n* = 261**	
Gestational age at birth (weeks)	40.05 ± 1.47	40.40 ± 1.09	38.01 ± 1.74	**
Weight live birth (grams)	3495.75 ± 495.38	3608.61 ± 419.09	2840.67 ± 384.17	**
Gender (% girls)	51.40	51.30	52.10	
Hair wash frequency	2.14 ± 0.81	2.13 ± 0.82	2.25 ± 0.74	*
Time since last wash	2.09 ± 0.83	2.09 ± 0.83	2.06 ± 0.84	
Use of hair product (% no)	75.90	77.20	68.00	**
Use of medication (% no)	91.80	92.20	89.50	
BMI child age 5	15.98 ± 1.56	16.02 ± 1.55	15.74 ± 1.62	*
Age child at hair sampling (months)	72.17 ± 4.92	72.13 ± 5.00	72.37 ± 4.43	
Hair colour (pigmentation)	2.37 ± 0.66	2.35 ± 0.66	2.50 ± 0.68	**
Sun hours	166.69 ± 76.57	166.96 ± 76.67	165.16 ± 76.12	
Disease sumscore	0.39 ± 0.72	0.39 ± 0.71	0.42 ± 0.76	
Allergy sumscore	0.14 ± 0.57	0.15 ± 0.58	0.12 ± 0.52	
CBCL externalizing sumscore	7.17 ± 6.25	7.05 ± 6.25	7.90 ± 6.21	
Hair cortisol pg/mg	4.85 ± 15.67	4.80 ± 15.92	5.13 ± 14.16	
Educational level mother	3.95 ± 1.01	3.98 ± 0.99	3.76 ± 1.06	**
Net month income of household	7.70 ± 2.66	7.78 ± 2.63	7.19 ± 2.77	**
Number of children in household	2.19 ± 0.75	2.20 ± 0.74	2.14 ± 0.80	
Ethnicity child				
% Dutch	66.60	67.60	60.90	
% other western	9.00	8.90	9.60	
% nonWestern	24.40	23.50	29.50	
Marital status mother at age 5 (% married/living together)	89.70	90.40	85.90	*
Maternal harsh parenting	2.14 ± 1.96	2.12 ± 1.94	2.30 ± 2.09	

Values presented are Mean ± SD, or valid percentages. The number of missings varies across variables. * = $p < .05$, ** = $p < .01$

Non-response analyses were performed to compare children who were included in the analysis (*n* = 1,776) with children who participated in hair collection but were excluded from the analysis due to missing data on (other) main variables (*n* = 1,794). Among included children, more mothers had a partner (89.7% vs. 78.8 %), mothers were higher educated (3.95 ± 1.01 vs. 3.21 ± 1.19), family monthly income was higher (7.70 ± 2.66 vs. 5.82 ± 2.96), more children had a Dutch ethnicity (66.6 % vs. 37.6%), average birth weight was higher (3495.75 ± 495.38 vs. 3300.53 ± 601.68), gestational age at birth was slightly higher (40.04 ± 1.47 vs. 39.54 ± 2.07), hair cortisol levels were lower 4.85 ± 15,67 vs. 5,75 ± 18.50)

and children were younger at hair sampling (72.17 ± 4.91 vs. 77.59 ± 9.44). More trivial differences are presented in Supplementary Table 1.

MEASURES

Mild perinatal adversity

Information about birth weight and gestational age were obtained from medical records completed by community midwives and obstetricians. Children were assigned to a group with mild perinatal adversity or a group without mild perinatal adversity (following Van der Kooy-Hofland et al., 2012). Criteria for assignment to the group with mild perinatal adversities were late preterm birth (gestational age at birth of 34-37 weeks, 6 days) or birth weight between the 2.5th and 10th percentile for full term gestational age (small for gestational age). Percentiles for the latter selection criterion were calculated based on all singleton children of the total Generation R Study with a gestational age at birth of at least 38 weeks (n = 8,348). The 2.5th and 10th percentiles were 2605,00 and 2890,00 grams. The group with mild perinatal adversities consisted of 261 children and the group without mild perinatal adversities consisted of 1515 children. Sample characteristics for the two groups are presented in Table 1. The two groups were compared using independent t-tests and Pearson's Chi square tests, showing differences on the following variables (*p*s <.05*)*: gestational age, birth weight, hair wash frequency, use of hair product, BMI, hair colour, maternal educational level, and parental marital status.

Maternal harsh parenting

Data on maternal harsh parenting was obtained by self-report questionnaire when the children were 3 years old (36.6 ± 1.1 months). In a previous study of the same cohort (Jansen et al., 2012), six items of the Parent-Child Conflict Tactics scale (Straus, Hamby, Finkelhor, Moore, & Runyan, 1998) were selected based on factor analysis to constitute a harsh discipline scale. The scale consisted of the following items: 'shook my child', 'shouted or screamed angrily at my child', 'called my child names', 'threatened to give a slap, but I didn't do it', 'angrily pinched my child's arm', 'called my child stupid, lazy, or something like that'. Confirmatory factor analyses indicated good fit for the harsh parenting factor (for additional details, see Jansen et al., 2012). Mothers rated their use of discipline types during the past 2 weeks on a 6-point scale ranging from 'never' to 'five times or more'. The categories twice, three times, four times, and five times were combined because of very low prevalence rates. This resulted in three categories: *never* (0), *once* (1), and *twice or more* (2). A harsh parenting sum score was calculated by summing the six items, yielding a score ranging from 0 to 12 with higher scores reflecting higher frequencies of harsh parenting behaviors. The distribution of the harsh parenting scores was skewed; sum scores were log transformed to approach a normal distribution.

Hair glucocorticoid measurement

During the lab visit at child age 6 (72.17 ± 4.92 months), hair samples of approximately 100 strands were cut from the posterior vertex using small surgical scissors, as close to the scalp as possible. Hair

locks were then taped to a piece of paper with the scalp end marked, and stored in an envelope at room temperature until further analyses. Cortisol was measured as described previously (Noppe, De Rijke, Dorst, Van den Akker, & Van Rossum, 2015), with the exception that hair samples were minced by hand using small surgical scissors, instead of using 1cm segments. Briefly, the proximal 3 cm of hair samples were weighed using an electrical scale and minced. Hair samples were then washed in LC-grade isopropanol for 2 minutes at room temperature, and left to dry for at least 2 days. Deuterium labeled cortisol and cortisone were added prior to extraction. Extraction was performed using LC-grade methanol (MeOH), for 18 hours at 25°C, in a gently shaking water basin. The extract was then transferred to a glass tube, centrifuged at 4300G, and evaporated to dryness at 37°C under a constant flow of N^2. After reconstitution in 1mL 2% LC-grade MeOH, the extract was loaded on an off-line solid phase extraction plate (HLB Oasis 96-well SPE plate, Waters Chromatography), washed with 1mL 30% LC-grade MeOH, and eluted twice in 300µL 100% LC-grade MeOH. The extract was then evaporated to dryness at 50°C under a constant flow of N^2 and stored at 4°C until further analysis. Prior to analysis, the samples were reconstituted in 100µL eluens, vortexed, and analysed using liquid chromatography tandem mass spectrometry (LC-MS/MS) (Xevo TQS, Waters Chromatography). The distribution of hair cortisol concentrations was highly skewed, therefore the values were log transformed to approach a normal distribution.

Additional measures for the main analysis

Analyses were adjusted for a set of covariates based on previous studies on hair cortisol (e.g. Dettenborn, Tietze, Kirschbaum, & Stalder, 2012; Noppe et al., 2015; Rippe et al., in prep). Data on hair washing frequency, time since the last wash, the use of hair products on the day of sampling, medication, BMI, and age of the child were obtained at the time of hair sampling. Hair colour was obtained by parent-report at child age 6. In case parent report was missing and photographs of the lab visit were available, hair colour (red / blond / brown / black) was coded by two raters. For reference, 50 additional subjects were coded to compare rater vs. parent report. To assess multirater agreement, Krippendorff Alpha (Hayes & Krippendorff, 2007; Krippendorff, 1980) was used. Comparisons were made for the two independent coders and between rating of the coders and hair colour as reported by the parents. Intercoder reliability was high for the two coders (0.79) and somewhat lower with parent report (0.69). The number of sun hours in the month of hair sampling was obtained from the KNMI (using www.zonurencalculator.nl) as a covariate, in order to correct for potential seasonal effects on hair growth (Randall & Ebling, 1991) and cortisol levels (Maes et al., 1997; Persson et al., 2008). Sum scores for disease and allergy were constructed from questionnaires completed at child age 6 (for more details, see Rippe et al., in prep). Both sum scores were highly skewed and hence log transformed to approach normality. Information on educational level of the mother, income, number of children (to be supported from this income), marital status, and ethnicity of the child was obtained using questionnaires at child age 6. Educational level was coded as follows: primary school, secondary phase 1 (lower vocational training, intermediate general school, <4 years of general secondary school), secondary phase 2 (>3 years of general secondary school; intermediate vocational training, 1st-year higher vocational training), higher phase 1 (higher vocational training, Bachelor's degree) and higher

phase 2 (higher academic education, PhD). Income was defined as the total net month income of the household. Marital status was dichotomized into 'married, registered partnership or living together' versus 'no partner'. Ethnicity of the child was classified into the categories 'Dutch', 'Western', and 'Non-Western', in accordance with the criteria of Statistics Netherlands (2004).

We also controlled for child externalizing behavior, because of previously described associations between externalizing behavior and cortisol levels (Alink et al., 2008) and harsh parenting (Mackenbach et al., 2014). Externalizing behavior of the child was assessed at five years of age (70.3 ± 4.2 months). The primary caregiver filled out a Dutch version of the Child Behavior Checklist (CBCL/1.5-5) (Achenbach & Rescorla, 2000), a widely used questionnaire with 99 items concerning the child's behavior in the previous two months. The broadband scale "Externalizing" comprises item scores on the subscales aggressive behavior and attention problems scales. Higher scores indicated more problems. Internal consistency of the CBCL externalizing scale was $\alpha = .90$.

Additional measures used for sensitivity analyses

Additional measures were used for follow-up sensitivity analyses. Reports of preeclampsia, pregnancy-induced hypertension or pre-existing hypertension during the pregnancy were obtained from medical records, completed by community midwives and obstetricians. Preeclampsia was defined according to the International Society for the Study of Hypertension in Pregnancy criteria (Brown et al., 2001).

Analyses

Outlying values for cortisol, age at the lab visit, BMI and externalizing behavior (defined as values outside the 3*IQR range, Cooper, Gulen, & Schill, 2008) were winsorized (Tabachnick & Fidell, 2012). Because of missing data on frequency of hair washing (3.6%), time since hair was last washed (3.9%), use of hair product (3.7%), medication use (3.8%), hair colour (0.1%), sun hours (0.2%), disease (7.8%), allergy (7.8%), educational level of the mother (6.1%), income (10.6%), number of children (7.0%), marital status mother (6.4%), ethnicity (0.2%) and externalizing behavior (6.3%), imputed data sets were generated. Missing data were imputed with the predictive mean matching (Markov chain Monte Carlo) method with 10 imputations and 10 iterations in IBM SPSS Statistics, version 21.

A hierarchical linear regression analysis was conducted to test the interaction effect of mild perinatal adversity and maternal harsh parenting on cortisol levels. In the regression equation, all covariates were included in the first step, followed by main effects of maternal harsh parenting and mild perinatal adversity in the second step. The interaction between maternal harsh parenting and mild perinatal adversity was included in the third step. Additionally, we tested whether gender played a moderating role, since previous studies indicated that boys might be more susceptible to environmental effects compared to girls (Kraemer, 2000; Mileva-Seitz et al., 2015), by including two- and three-way interactions of gender, harsh parenting and mild perinatal adversity. Interaction terms were computed after centering of the principal variables. Non-significant interaction terms were removed from the model. All statistics were pooled by SPSS, except for the standardized regression coefficient β, R^2 and change in R^2, for which we calculated the average values over the 10 imputed datasets.

In case of significant interaction effects, additional stratified analyses were conducted to investigate associations per subgroup. The analyses were conducted in separate imputed data sets and subsequently pooled to obtain an overall result based on the imputations. Additionally, significant interactions were examined in more detail by performing additional analyses following Widaman et al. (2012). Re-parameterized regression analyses were conducted to obtain point and interval estimates of the cross-over point of the interaction, to distinguish between an ordinal interaction, consistent with a diathesis stress model, and a disordinal interaction, consistent with a differential susceptibility model.

In case of significant interaction effects with mild perinatal adversity, sensitivity analyses were conducted. Our first sensitivity analysis was carried out to rule out the possibility that the effects were due to inclusion of children with a gestational age of more than 42 weeks. In a second sensitivity analysis, we excluded children with preeclampsia complications during pregnancy, to examine the moderating effect of mild perinatal adversity (late preterm birth and low birth weight) not caused by preeclampsia. Per sensitivity analysis, the final regression model was run without these selected children and results were compared to the original results.

RESULTS

Descriptive statistics are presented in Table 1. Collinearity checks in the regression analyses yielded no indication of problematic associations among predictors. Harsh parenting sum scores did not significantly differ between groups with and without mild perinatal adversity.

Harsh parenting and hair cortisol levels: moderation by mild perinatal adversity?

Hierarchical regression analysis showed that all two- and three-way interactions including gender were not significant; they were therefore removed from the model. Pooled results of the final hierarchical regression model for the 10 generated datasets are presented in Table 2. Time since the hair was last washed ($\beta = 0.07$, $p < .05$; more recent last wash related to lower cortisol), use of medication ($\beta = 0.07$, $p < .01$; higher cortisol levels in case of medication use), BMI ($\beta = 0.07$, p .01; higher BMI related to higher cortisol), hair colour ($\beta = 0.07$, $p < .05$; darker hair colour related to higher cortisol), disease ($\beta = 0.05$, $p < .05$; higher disease score related to higher cortisol), net month income ($\beta = -0{,}09$, $p < .05$; lower net income related to higher cortisol), number of children in the household ($\beta = 0.05$, $p < .05$; more children related to higher cortisol) and ethnicity (lower cortisol levels in Dutch children compared to other western ($\beta = 0.06$, $p < .01$) and non-western ($\beta = 0.14$, $p < .001$) children) were significant predictors of hair cortisol levels in the first step. Gender also significantly predicted hair cortisol levels ($\beta = 0.09$, $p < .001$), with higher cortisol levels for boys. Neither maternal harsh parenting nor mild perinatal adversity predicted cortisol levels. However, an interaction effect of maternal harsh parenting and mild perinatal adversity was found ($\beta = 0.05$, $p < .05$). Analyses in the complete (not-imputed) data set ($n = 1429$) yielded very similar results, only the effects of hair colour and disease did not reach significance. A plot of the interaction effect is presented in Figure 1.

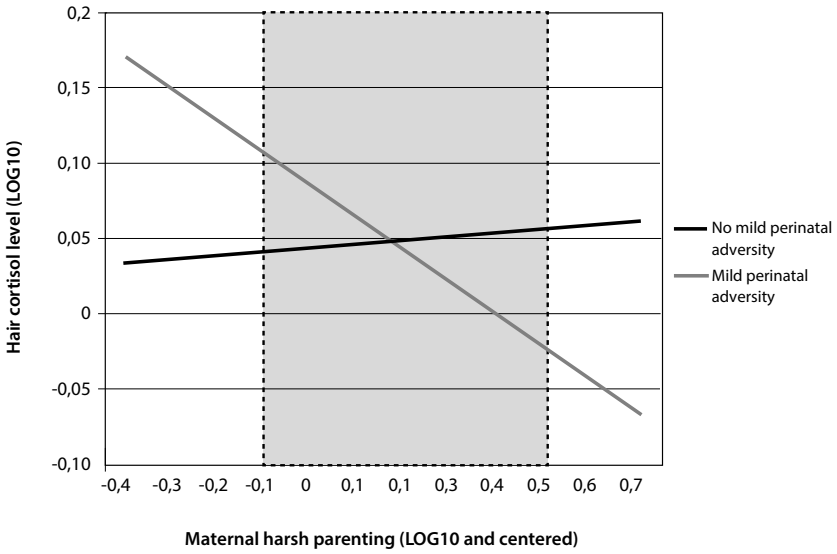

Figure 1 | The interaction effect of maternal harsh parenting and mild perinatal adversity on hair cortisol levels

Note. Figure is based on B's from the final model, adjusted for all other variables in the model. The grey area represents the 95% confidence interval for the cross-over point of the interaction.

To further explore this interaction effect, partial correlations between hair cortisol and maternal harsh parenting, controlling for all other main effects in the model, were computed for the groups with and without mild perinatal adversity separately. In the group without mild perinatal adversity, maternal harsh parenting was unrelated to hair cortisol (r = .02, p(range) = .391-.529. In the group with mild perinatal adversity, a negative association was found (r = -0.13, p(range) = .026-.078). In children with mild perinatal adversity, higher scores on maternal harsh parenting were associated with lower hair cortisol levels. A Fisher r-to-z transformation showed that the correlations for the two groups were significantly different (z = -2.22, p = .026).

Table 2 | Final model for cortisol

	B	SE	95% Confidence Interval for B	β [a]	t	*p*-value	R^{2a}	ΔR^{2a}
Step 1							0.08	0.08**
(Constant)	0.05	0.31	[-0.56, 0.65]		0.15	.879		
Frequency hair washing	-0.02	0.02	[-0.05, 0.02]	-0.03	-0.97	.330		
Time since last wash	**0.04**	**0.02**	**[0.01, 0.07]**	**0.07**	**2.57**	**.010**		
Use of hair product	-0.01	0.03	[-0.06, 0.05]	-0.01	-0.21	.837		
Use of medication	**0.12**	**0.04**	**[0.04, 0.21]**	**0.07**	**2.86**	**.004**		
BMI age 5	**0.02**	**0.01**	**[0.01, 0.04]**	**0.07**	**2.73**	**.006**		
Child age at hair sampling	-0.01	0.00	[-0.01, 0.00]	-0.03	-1.25	.213		
Hair colour (pigmentation)	**0.05**	**0.02**	**[0.01, 0.10]**	**0.07**	**2.55**	**.011**		
Sun hours	0.00	0.00	[0.00, 0.00]	0.01	0.26	.797		
Disease	**0.15**	**0.07**	**[0.01, 0.28]**	**0.05**	**2.16**	**.031**		
Allergy	0.02	0.10	[-0.18, 0.21]	0.00	0.17	.867		
Educational level mother	0.01	0.02	[-0.02, 0.04]	0.01	0.44	.657		
Net month income of household	**-0.02**	**0.01**	**[-0.03, 0.00]**	**-0.09**	**-2.57**	**.010**		
Number of children in household	**0.03**	**0.02**	**[0.00, 0.07]**	**0.05**	**2.12**	**.034**		
Marital status parents age 5	0.01	0.04	[-0.08, 0.09]	0.01	0.19	.847		
Ethnicity Dutch vs. other western	**0.11**	**0.04**	**[0.03, 0.19]**	**0.06**	**2.71**	**.007**		
Ethnicity Dutch vs. nonwestern	**0.16**	**0.03**	**[0.10, 0.23]**	**0.14**	**4.73**	**.000**		
CBCL externalizing age 5	0.00	0.00	[-0.01, 0.00]	-0.02	-0.82	.411		
Gender	**0.04**	**0.01**	**[0.02, 0.06]**	**0.09**	**3.64**	**.000**		
Step 2							0.08	0.00
Maternal harsh parenting (LOG10)	-0.01	0.04	[-0.09, 0.08]	0.00	-0.12	.903		
Mild perinatal adversity yes/no	0.02	0.02	[-0.01, 0.05]	0.03	1.42	.156		
Step 3							0.08	0.00*
maternal harsh parenting * mild perinatal adversity	**-0.12**	**0.06**	**[-0.23, 0.00]**	**-0.05**	**-2.03**	**.042**		

[a] average taken from the final regression models of the 10 imputed datasets

Note. Statistics are taken from the final models.

Testing for differential susceptibility: distinguishing ordinal from disordinal interaction

Additional analyses following Widaman et al. (2012) were performed to distinguish whether the interaction effect of maternal harsh parenting and mild perinatal adversity was ordinal or disordinal. First, a standard regression model, similar to the previous analysis, was fit to the data using nonlinear regression analyses. In a next step, a re-parameterized model was fitted to the data by centring the data around the cross-over point of the interaction. The mean point estimate of the cross-over point

Table 3 | Results for analyses following Widaman et al. (2012) in order to distinguish ordinal from disordinal interaction

Standard Parameterization					Re-parameterized model				
	Parameter	Estimate[a]	SE[a]	95% CI[a]		Parameter	Estimate[a]	SE[a]	95% CI[a]
Constant	B^*_0	0.05	0.31	[-0.55, 0.65]	Constant	A^*_0	0.05	0.31	[-0.56, 0.65]
Maternal harsh parenting	B^*_1	-0.01	0.04	[-0.09, 0.08]	Maternal harsh parenting for group with mild perinatal adversity	B^*_1	-0.20	0.11	[-0.42, 0.01]
Mild perinatal adversity yes/no	B^*_2	0.02	0.02	[-0.01, 0.05]	Centering constant (cross-over point)	C*	0.19	0.16	[-0.12, 0.51]
Maternal harsh parenting * mild perinatal adversity	B^*_3	-0.12	0.06	[-0.23, 0.00]	Maternal harsh parenting for group without mild perinatal adversity	B^*_2	0.03	0.05	[-0.06, 0.12]
Frequency hair washing	B^*_4	-0.02	0.02	[-0.05, 0.02]	Frequency hair washing	B^*_4	-0.02	0.02	[-0.05, 0.02]
Time since last wash	B^*_5	0.04	0.02	[0.01, 0.07]	Time since last wash	B^*_5	0.04	0.02	[0.01, 0.07]
Use of hair product	B^*_6	-0.01	0.03	[-0.06, 0.05]	Use of hair product	B^*_6	-0.01	0.03	[-0.06, 0.05]
Use of medication	B^*_7	0.12	0.04	[0.04, 0.20]	Use of medication	B^*_7	0.12	0.04	[0.04, 0.20]
BMI age 5	B^*_8	0.02	0.01	[0.01, 0.04]	BMI age 5	B^*_8	0.02	0.01	[0.01, 0.04]
Child age at hair sampling	B^*_9	-0.01	0.00	[-0.01, 0.00]	Child age at hair sampling	B^*_9	-0.01	0.00	[-0.01, 0.00]
Hair colour (pigmentation)	B^*_{10}	0.05	0.02	[0.01, 0.10]	Hair colour	B^*_{10}	0.05	0.02	[0.01, 0.10]
Sun hours	B^*_{11}	0.00	0.00	[0.00, 0.00]	Sun hours	B^*_{11}	0.00	0.00	[0.00, 0.00]
Disease	B^*_{12}	0.15	0.07	[0.02, 0.28]	Disease	B^*_{12}	0.15	0.07	[0.02, 0.28]
Allergy	B^*_{13}	0.02	0.10	[-0.17, 0.21]	Allergy	B^*_{13}	0.02	0.10	[-0.17, 0.21]
Educational level mother	B^*_{14}	0.01	0.01	[-0.02, 0.03]	Educational level mother	B^*_{14}	0.01	0.01	[-0.02, 0.03]
Net month income of household	B^*_{15}	-0.02	0.01	[-0.03, 0.00]	Net month income of household	B^*_{15}	-0.02	0.01	[-0.03, 0.00]
Number of children in household	B^*_{16}	0.03	0.02	[0.00, 0.06]	Number of children in household	B^*_{16}	0.03	0.02	[0.00, 0.06]
Marital status parents age 5	B^*_{17}	0.01	0.04	[-0.07, 0.09]	Marital status parents age 5	B^*_{17}	0.01	0.04	[-0.07, 0.09]
Ethnicity Dutch vs. other western	B^*_{18}	0.11	0.04	[0.03, 0.19]	Ethnicity Dutch vs. other western	B^*_{18}	0.11	0.04	[0.03, 0.19]
Ethnicity Dutch vs. nonwestern	B^*_{19}	0.16	0.03	[0.09, 0.23]	Ethnicity Dutch vs. nonwestern	B^*_{19}	0.16	0.03	[0.09, 0.23]
CBCL externalizing age 5	B^*_{20}	0.00	0.00	[-0.01, 0.00]	CBCL externalizing age 5	B^*_{20}	0.00	0.00	[-0.01, 0.00]
Gender	B^*_{21}	0.04	0.01	[0.02, 0.06]	Gender	B^*_{21}	0.04	0.01	[0.02, 0.06]

CI = confidence interval. Mean-centered variables were used. [a] average taken of the 10 imputed datasets

Table 4 | Results of the original analysis and sensitivity analyses

	Original results			Sensitivity analyses					
				Gestational age >42w			Preeclampsia		
	n = 1776			*n* = 1679			*n* = 1442		
	B	β [a]	*p*-value	B	β [a]	*p*-value	B	β [a]	p-*value*
Step 1									
(Constant)	0.05		.879	0.02		.952	0.04		.916
Frequency hair washing	-0.02	-0.03	.330	-0.01	-0.02	.580	-0.02	-0.04	.207
Time since last wash	0.04	0.07	.010	0.04	0.07	.013	0.03	0.06	.076
Use of hair product	-0.01	-0.01	.837	0.00	0.00	.944	0.01	0.01	.743
Use of medication	0.12	0.07	.004	0.11	0.07	.008	0.08	0.04	.104
BMI age 5	0.02	0.07	.006	0.02	0.06	.018	0.03	0.08	.002
Child age at hair sampling	-0.01	-0.03	.213	0.00	-0.02	.319	-0.01	-0.04	.121
Hair colour (pigmentation)	0.05	0.07	.011	0.04	0.06	.043	0.05	0.08	.017
Sun hours	0.00	0.01	.797	0.00	0.00	.993	0.00	0.01	.616
Disease	0.15	0.05	.031	0.15	0.05	.029	0.19	0.07	.012
Allergy	0.02	0.00	.867	0.01	0.00	.948	0.05	0.01	.688
Educational level mother	0.01	0.01	.657	0.01	0.01	.638	0.02	0.04	.274
Net month income of household	-0.02	-0.09	.010	-0.02	-0.09	.015	-0.02	-0.09	.032
Number of children in household	0.03	0.05	.034	0.04	0.06	.028	0.05	0.08	.002
Marital status parents age 5	0.01	0.01	.847	0.01	0.01	.839	0.02	0.01	.748
Ethnicity Dutch vs. other western	0.11	0.06	.007	0.12	0.07	.004	0.06	0.04	.152
Ethnicity Dutch vs. nonwestern	0.16	0.14	.000	0.17	0.15	.000	0.16	0.15	.000
CBCL externalizing age 5	0.00	-0.02	.411	0.00	-0.01	.650	0.00	-0.02	.468
Gender	0.04	0.09	.000	0.04	0.08	.001	0.05	0.10	.000
Step 2									
Maternal harsh parenting (LOG10)	-0.01	0.00	.903	-0.01	-0.01	.821	0.00	0.00	.994
Mild perinatal adversity yes/no	0.02	0.03	.156	0.02	0.03	.207	0.03	0.04	.091
Step 3									
maternal harsh parenting * mild perinatal adversity	-0.12	-0.05	.042	-0.11	-0.04	.072	-0.14	-0.06	.026

[a] average taken of the 10 imputed datasets

across the 10 imputed data-sets was 0.19 and the mean 95 % CI interval was [-0.12-0.51] (Table 3). Both the point and interval estimates of the cross-over point of the interaction fell within the observed range of harsh parenting (range: -0.42-0.70, see Figure 1) indicating a disordinal cross-over interaction in which the two regression lines cross in the middle of the distribution of the predictor variable (harsh parenting) consistent with a differential susceptibility model.

Sensitivity analyses

Two sensitivity analyses were conducted in the imputed data sets. Children were excluded (i) if they had a gestational age of over 42 weeks (n = 97; 3 from the group with mild perinatal adversity, 94 from the group without mild perinatal adversity) and (ii) if preeclampsia or hypertension complications were present during the pregnancy or data on pregnancy complications were missing (n = 334; 61 from the group with mild perinatal adversity, 273 from the group without mild perinatal adversity). Results of the sensitivity analyses are presented in Table 4. For sensitivity analysis 1, the beta for the interaction remained similar to the original analysis (β = -0.04 vs. β = -0.05), but the interaction effect was no longer significant (p = .072), probably because of the lower sample size. The other sensitivity analysis showed unchanged results, with a significant interaction effect of maternal harsh parenting and mild perinatal adversity.

DISCUSSION

In this study we tested mild perinatal adversity as a susceptibility marker moderating the association between maternal harsh parenting at age 3 and children's hair cortisol levels at age 6. Consistent with our hypothesis, we found that mild perinatal adversity moderated the association between maternal harsh parenting with hair cortisol levels. A negative association was found between maternal harsh parenting and cortisol levels, but only in children with mild perinatal adversity. These children showed lower cortisol levels when they experienced more maternal harsh parenting, and higher cortisol levels in the absence of harsh parenting, compared with children without mild perinatal adversity. Additional analyses following Widaman et al. (2012) indicated that the interaction was disordinal, consistent with the differential susceptibility model.

The lower hair cortisol levels in the children with both greater harsh parenting and mild perinatal adversity are consistent with a down-regulation of the HPA axis as a result of chronic stress (Fries, Hesse, Hellhammer, & Hellhammer, 2005; Gunnar & Vazquez, 2001). In a meta-analysis, Miller et al. (2007) showed that HPA axis activity is elevated at the onset of stress, but decreases with time. Moreover, at the age of the children in our sample, externalizing behavior is associated with lower basal cortisol levels (hypoactivity) (Alink et al., 2008). Our results of low cortisol levels in case of harsh parenting are thus not inconsistent with meta-analytically established correlates of low cortisol levels. In the absence of harsh parenting, children with mild perinatal adversity showed the highest hair cortisol levels. We speculate that higher cortisol levels reflect an elevated biological sensitivity to the environment. In light of Boyce's concept of biological sensitivity to context, a heightened reactivity to the environment would be beneficial in positive environments as it would open up opportunities for learning and adaptation (Boyce & Ellis, 2005). If heightened hair cortisol in these children is an indicator of overall greater reactivity to the environment, our results support a differential susceptibility model with mild perinatal adversity being a susceptibility marker, increasing susceptibility to both positive and negative environments.

Our results are consistent with findings by Van der Kooy-Hofland et al. (2012) that mild perinatal adversity moderates the effects of an educational experiment changing exposure to literacy experiences and more generally was considered to be a susceptibility factor. In another study, children with both low and very low birth weight were more susceptible than children with normal birth weight to the adverse effects of low sensitive parenting in association with academic achievement at age 6. However, these same children were *not* more susceptible to the positive effects of high-sensitive parenting (Jaekel, Pluess, Belsky, & Wolke (2015). Shah, Robbins, Coelho, and Poehlmann (2013) reported that very preterm children (gestational age <30 weeks of gestation) had the lowest cognitive scores at age 3 in the context of more negative parenting, but the highest in the context of less negative parenting. For late pre-term children (gestational age 34-36 weeks, 6 days) cognitive outcomes were not associated with negative parenting. Thus these results were consistent with differential susceptibility, but pointed to the very preterm, rather than the late-preterm children, as the most susceptible group. It is important to note, however, that a full term group was not included as a reference group. In addition, both Jaekel et al. (2015) and Shah et al. (2013) used measures of perinatal adversity that were only partially overlapping with the one Van der Kooy-Hofland et al. (2012) and we used in the current study. Future studies investigating perinatal adversity as a susceptibility marker might include a range from extreme to no prenatal adversity, and should examine in more detail to what extent perinatal adversities influence susceptibility to the environment.

Prenatal stress may program increased developmental plasticity to enhance adaptation to the postnatal environment through the influence on physiological and behavioral markers of susceptibility (Ellis et al., 2011; Pluess & Belsky, 2011). (Mild) perinatal adversity has indeed been shown to be associated with increased stress reactivity, for example reflected in higher basal cortisol levels and increased cortisol reactivity (Economides et al., 1988; Jones et al., 2006), and with increased negative emotionality (Meier, Wolke, Gutbrod, & Rust, 2003; Pluess & Belsky, 2011). The degree of prenatal programming could in turn differ across individuals based on their genetic makeup. For instance, maternal anxiety during pregnancy predicted child negative emotionality at 6 months, but only in infants carrying one or more copies of the 5-HTTLPR short allele (Pluess et al., 2011). In our study, we did not find a main effect of mild perinatal adversity on cortisol levels at 6. This might be due to the fact that we used hair cortisol, a measure for chronic stress, or in relatively typical populations maybe better: heightened activity levels – whereas previous studies (e.g. Jones et al., 2006) used momentary cortisol levels. Nevertheless, our results showing in the case of low harsh parenting higher cortisol levels in children with versus without mild perinatal adversity suggest elevated biological sensitivity to the environment as conceptualized by Boyce and Ellis (2005).

Some methodological considerations should be taken into account. A strength of this study is the use of a large subsample from a longitudinal population-based cohort study. Moreover, hair cortisol as a biomarker for chronic stress has been shown to be a stable and objective measure of a new dimension of HPA axis activity: cumulative cortisol levels over longer time periods. This is a promising measure, for instance in the investigation of effects of parental caregiving on stress levels, in addition to more traditional cortisol measures mostly reflecting short term cortisol reactivity or daily patterns (Hostinar & Gunnar, 2013). A third strength is the method used for the assessment of hair cortisol. Most studies

reporting on hair cortisol concentrations relied on immunoassays, such as ELISA. However, these assays have several disadvantages including cross-reactivity and the limitation to single component measurement per assay. We assessed hair cortisol using a LC-MS/MS assay, which is preferable because of superior specificity and sensitivity (Noppe et al., 2015).

This study also has some limitations. A first limitation is the long time lag between the harsh parenting measure, assessed at age 3, and hair cortisol assessed at age 6. Cortisol levels are influenced by many physical and environmental factors. Therefore, we cannot rule out that other experiences contributed to the results. However, a recent study showed that levels of harsh parenting at age 3 moderated the effects of later traumatic events on cortisol reactivity at age 10: the experience of more traumatic events was associated with greater cortisol reactivity, but only in the case of low -not high- levels of early harsh parenting (Jaffee et al., 2015). These results underscore the importance of early experiences in shaping HPA axis activity. Furthermore, assessing harsh parenting we did not measure optimal parenting as the absence of harsh parenting is not the same as the presence of great caregiving. In future studies measures for harsh and sensitive parenting should be included to cover a broader range of environmental influences. Finally, we assessed harsh parenting using a self-report questionnaire. Response biases such as social desirability may have resulted in an underestimation of harsh parenting behavior. However, since misclassification is more likely in parents who reported no harsh parenting than in parents who did report harsh parenting behavior, underreporting would result in an underestimation of the effects rather than an overestimation.

The current study provides further evidence that mild perinatal adversity may act as a susceptibility factor, moderating environmental effects for the better and for the worse (Ellis et al., 2011). Maternal harsh parenting was associated with hair cortisol levels in children with a history of mild perinatal adversity. Our results once more emphasize the importance of the early caregiving environment in the development of the HPA axis, although the influence may be dependent on differential susceptibility of children to the environment.

REFERENCES

Achenbach, T. M., & Rescorla, L. A. (2000). *Manual for the ASEBA Preschool forms & profiles.* Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families.

Adam, E. K., Klimes-Dougan, B., & Gunnar, M. R. (2007). Social regulation of the adrenocortical response to stress in infants, children, and adolescents: Implications for psychopathology and education. In D. Coch, G. Dawson, & K. W. Fischer (Eds.) *Human behavior, learning, and the developing brain: Atypical development* (pp. 264-304). New York: The Guilford Press.

Alink, L. R. A., Van IJzendoorn, M. H., Bakermans-Kranenburg, M. J., Mesman, J., Juffer, F., & Koot, H. M. (2008). Cortisol and externalizing behavior in children and adolescents: Mixed meta-analytic evidence for the inverse relation of basal cortisol and cortisol reactivity with externalizing behavior. *Developmental Psychobiology, 50,* 427-450.

Belsky, J., Bakermans-Kranenburg, M. J., & Van IJzendoorn, M. H. (2007). For better and for worse – Differential susceptibility to environmental influences. *Current Directions in Psychological Science*, *16,* 300-304.

Boyce, W. T., Chesney, M., Alkon, A., Tschann, J. M., Adams, S., Chesterman, B., … Wara, D. (1995). Psychobiologic reactivity to stress and childhood respiratory illnesses: Results of two prospective studies. *Psychosomatic Medicine, 57,* 411-422.

Boyce, W. T., & Ellis, B. J. (2005). Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. *Development and Psychopathology, 17,* 271-301.

Brown, M. A., Lindheimer, M. D., De Swiet, M., Van Assche, A., & Moutquin, J. M. (2001). The classification and diagnosis of the hypertensive disorders of pregnancy: Statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertension in Pregnancy, 20*, IX-XIV.

Chang, L., Schwartz, D., Dodge, K. A., & McBride-Chang, C. (2003). Harsh parenting in relation to child emotion regulation and aggression. *Journal of Family Psychology, 17,* 598-606.

Cooper, M. J., Gulen, H., & Schill, M. J. (2008) Asset growth and the cross-section of stock returns. *The Journal of Finance, 63,* 1609-1651.

De Kloet, E. R., Joëls, M., & Holsboer, F. (2005). Stress and the brain: From adaptation to disease. *Nature Reviews Neuroscience*, *6,* 463-475.

Dettenborn, L., Tietze, A., Kirschbaum, C., & Stalder, T. (2012). The assessment of cortisol in human hair: Associations with sociodemographic variables and potential confounders. *Stress, 15,* 578-588.

Economides, D. L., Nicolaides, K. H., Linton, E. A., Perry, L. A., & Chard, T. (1988). Plasma cortisol and adrenocorticotropin in appropriate and small for gestational age fetuses. *Fetal Therapy*, *3,* 158-164.

Ellis, B. J., Boyce, W. T., Belsky, J., Bakermans-Kranenburg, M. J., & Van IJzendoorn, M. H. (2011). Differential susceptibility to the environment: An evolutionary-neurodevelopmental theory. *Development and Psychopathology, 23,* 7-28.

Fries, E., Hesse, J., Hellhammer, J., & Hellhammer, D. H. (2005). A new view on hypocortisolism. *Psychoneuroendocrinology*, *30,* 1010-1016.

Gunnar, M. R., & Donzella, B. (2002). Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology, 27,* 199-220.

Gunnar, M. R., & Quevedo, K. (2007). The neurobiology of stress and development. *Annual Review of Psychology, 58,* 145-173.

Gunnar, M. R., & Vazquez, D. M. (2001). Low cortisol and a flattening of expected daytime rhythm: Potential indices of risk in human development. *Development and Psychopathology*, *13,* 515-538.

Hastings P. D., Ruttle, P. L., Serbin, L. A., Mills, R. S. L., Stack, D. M., & Schwartzman, A. E. (2011). Adrenocortical responses to strangers in preschoolers: Relations with parenting, temperament, and psychopathology. *Developmental Psychobiology, 53,* 694-710.

Hayes, A. F., & Krippendorff, K. (2007). Answering the call for a standard reliability measure for coding data. *Communication Methods and Measures, 1,* 77-89.

Hostinar, C. E., & Gunnar, M. R. (2013). Future directions in the study of social relationships as regulators of the HPA axis across development. *Journal of Clinical Child & Adolescent Psychology, 42,* 564-575.

Hostinar, C. E., Sullivan, R. M., & Gunnar, M. R. (2014). Psychobiological mechanisms underlying the social buffering of the hypothalamic-pituitary-adrenocortical axis: A review of animal models and human studies across development. *Psychological Bulletin, 140,* 256-282.

Jaddoe, V. W. V., Van Duijn, C. M., Franco, O. H., Van der Heijden, A. J., Van IJzendoorn, M. H., De Jongste, J. C., … Hofman, A. (2012). The Generation R Study: Design and cohort update 2012. *European Journal of Epidemiology, 27,* 739-756.

Jaekel, J., Pluess, M., Belsky, J., & Wolke, D. (2015). Effects of maternal sensitivity on low birth weight children's academic achievement: A test of differential susceptibility versus diathesis stress. *Journal of Child Psychology and Psychiatry*, *56,* 693-701.

Jaffee, S. R., McFarquhar, T., Stevens, S., Ouellet-Morin, I., Melhuish E., & Belsky, J. (2015). Interactive effects of early and recent exposure to stressful contexts on cortisol reactivity in middle childhood. *Journal of Child Psychology and Psychiatry, 56,* 138-146.

Jansen, P. W., Raat, H., Mackenbach, J. P., Hofman, A., Jaddoe, V. W. V., Bakermans-Kranenburg, M. J. … Tiemeier, H. (2012). Early determinants of maternal and paternal harsh discipline: The Generation R Study. *Family Relations, 61,* 253–270.

Jones, A., Godfrey, K. M., Wood, P., Osmond, C., Goulden, P., & Philips, D. I. W. (2006). Fetal growth and the adrenocortical response to psychological stress. *The Journal of Clinical Endocrinology & Metabolism, 91,* 1868-1871.

Kirschbaum, C., & Hellhammer, D. H. (1989). Salivary cortisol in psychobiological research: An overview. *Neuropsychobiology, 22,* 150–169.

Kraemer, S. (2000) The fragile male. *British Medical Journal*, *321,* 1609-1612

Krippendorff, K. (1980). *Content analysis: An introduction to its methodology*. Beverly Hills, CA: Sage.

Larzelere, R. E. (2000). Child outcomes of nonabusive and customary physical punishment by parents: An updated literature review. *Clinical Child and Family Psychology Review*, *3*, 199 – 221.

Levine, S. (2001). Primary social relationships influence the development of the hypothalamic-pituitary-adrenal axis in the rat. *Physiology & Behavior, 73,* 255-260.

Mackenbach, J. D., Ringoot, A. P., Van der Ende, J., Verhulst, F. C., Jaddoe, V. W. V., Hofman, A., … Tiemeier, H. (2014). Exploring the relation of harsh parental discipline with child emotional and behavioral problems by using multiple informants. The Generation R study. *PLoS ONE, 9,* e104793.

Maes, M., Mommen, K., Hendrickx, D., Peeters, D., D'Hondt, P., Ranjan, R., ... Scharpé, S. (1997). Components of biological variation, including seasonality, in blood concentrations of TSH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers. *Clinical Endocrinology*, *46,* 587-598.

Manenschijn, L., Koper, J. W., Lamberts, S. W. J., & Van Rossum, E. F. C. (2011). Evaluation of a method to measure long term cortisol levels. *Steroids, 76,* 1032- 1036.

McLoyd, V. C., & Smith, J. (2002). Physical discipline and behavior problems in African American, European American, and Hispanic children: Emotional support as moderator. *Journal of Marriage and Family, 64,* 40-53.

Meier, P., Wolke, D., Gutbrod, T., & Rust, L. (2003). The influence of infant irritability on maternal sensitivity in a sample of very premature infants. *Infant and Child Development, 12,* 159–166.

Mileva-Seitz, V. R., Ghassabian, A., Bakermans-Kranenburg, M. J., Van den Brink, J. D., Linting, M., Jaddoe, V. W. V., …Van IJzendoorn, M.H. (2015). Are boys more sensitive to sensitivity? Parenting and executive function in preschoolers. *Journal of Experimental Child Psychology, 130,* 193-208.

Miller, G. E., Chen, E., & Zhou, E. S. (2007). If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychological Bulletin, 133,* 25-45.

Noppe, G., De Rijke, Y. B., Dorst, K., Van den Akker, E. L. T., & Van Rossum, E. F. C. (2015). LC-MS/MS based method for long-term steroid profiling in human scalp hair. *Clinical Endocrinology, 83, 162-166.*

Noppe, G., Van Rossum, E. F. C., Koper, J. W., Manenschijn, L., Bruining, G. J., De Rijke, Y. B., & Van den Akker, E. L. T. (2014). Validation and reference ranges of hair cortisol measurement in healthy children. *Hormone Research in Paediatrics*, *82,* 97-102.

Obradović, J. (2012). How can the study of physiological reactivity contribute to our understanding of adversity and resilience processes in development? *Development and Psychopathology, 24,* 371-387.

Pendry, P., & Adam, E. K. (2007). Associations between parents' marital functioning, maternal parenting quality, maternal emotion and child cortisol levels. *International Journal of Behavioral Development, 31,* 218-231.

Persson, R., Garde, A. H., Hansen, Å. M., Österberg, K., Larsson, B., Ørbæk, P., & Karlson, B. (2008). Seasonal variation in human salivary cortisol concentration. *Chronobiology International, 25,* 923-937.

Pluess, M., & Belsky, J. (2011). Prenatal programming of postnatal plasticity? *Development and Psychopathology, 23,* 29 –38.

Pluess, M., Velders, F. P., Belsky, J., Van IJzendoorn, M. H., Bakermans-Kranenburg, M. J., Jaddoe, V. W. V., … Tiemeier, H. (2011). Serotonin transporter polymorphism moderates effects of prenatal maternal anxiety on infant negative emotionality. *Biological Psychiatry*, *69,* 520-525.

Poulter, N. R., Chang, C. L., MacGregor, A. J., Snieder, H., & Spector, T. D. (1999). Association between birth weight and adult blood pressure in twins: Historical cohort study. *British Medical Journal*, *319,* 1330-1333.

Rippe, R. C. A., Noppe, G., Windhorst, D. A., Tiemeier, H., Van Rossum, E. F. C., Jaddoe, V. W. V., … Van den Akker, E. L. T. Hair on cortisol: Associations of socio-economic status, ethnicity, hair color, and other child characteristics with hair cortisol and cortisone. *Manuscript in preparation.*

Randall, V. A., & Ebling, F. J. G. (1991). Seasonal changes in human hair growth. *British Journal of Dermatology, 124,* 146-151.

Russell, E., Koren, G., Rieder, M., & Van Uum, S. (2012). Hair cortisol as a biological marker of chronic stress: Current status, future directions and unanswered questions. *Psychoneuroendocrinology, 37,* 589-601.

Sameroff, A. J. (1983). Developmental systems: Contexts and evolution. In P. Mussen (Ed.), *Handbook of child psychology* (Vol. 1, pp. 237–294). New York: Wiley.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews, 21,* 55-89.

Shah, P. E., Robbins, N., Coelho, R. B., & Poehlmann, J. (2013). The paradox of prematurity: The behavioral vulnerability of late preterm infants and the cognitive susceptibility of very preterm infants at 36 months post-term. *Infant Behavior and Development, 36,* 50-62.

Solomon, C. R., & Serres, F. (1999). Effects of parental verbal aggression on children's self-esteem and school marks. *Child Abuse & Neglect*, *23*, 339 – 351.

Stalder, T., & Kirschbaum, C. (2012). Analysis of cortisol in hair – State of the art and future directions. *Brain, Behavior, and Immunity, 26,* 1019-1029.

Statistics Netherlands (2004). *Allochtonen in Nederland 2004 [Foreigners in the Netherlands 2004].* Voorburg/ Heerlen: Statistics Netherlands.

Straus, M. A., & Field, C. J. (2003). Psychological aggression by American parents: National data on prevalence, chronicity, and severity. *Journal of Marriage and Family*, *65*, 795– 808.

Straus, M. A., Hamby, S. L., Finkelhor, D., Moore, D. W., & Runyan, D. (1998). Identification of child maltreatment with the Parent-Child Conflict Tactics Scales: Development and psychometric data for a national sample of American parents. *Child Abuse & Neglect, 22,* 249-270.

Tabachnick, B. G., & Fidell, L. S. (2012). *Using multivariate statistics* (6th edition). New York: Harper Collins.

Tarullo, A. R., & Gunnar, M.R. (2006). Child maltreatment and the developing HPA axis. *Hormones and Behavior, 50,* 632-639.

Teicher, M. H., Samson, J. A., Polcari, A., & McGreenery, C. E. (2006). Sticks, stones, and hurtful words: Relative effects of various forms of childhood maltreatment. *American Journal of Psychiatry*, *163*, 993 – 1000.

Tiemeier, H., Velders, F. P., Szekely, E., Roza, S. J. Dieleman, G., Jaddoe, V. W. V., …Verhulst, F. C. (2012). The Generation R Study: A review of design, findings to date and a study of the 5-HTTLPR by environmental interaction from fetal life onward. *Journal of the American Academy of Child and Adolescent Psychiatry, 51,* 1119- 1135.e7.

Vanaelst, B., Huybrechts, I., Bammann, K., Michels, N., De Vriendt, T., Vyncke, K., ... De Henauw, S. (2012). Intercorrelations between serum, salivary, and hair cortisol and child-reported estimates of stress in elementary school girls. *Psychophysiology, 49,* 1072-1081.

Van der Kooy-Hofland, V. A. C., Van der Kooy, J., Bus, A. G., Van IJzendoorn, M. H., & Bonsel, G. J. (2012). Differential susceptibility to early literacy intervention in children with mild perinatal adversities: Short- and long-term effects of a randomized control trial. *Journal of Educational Psychology, 104,* 337-349.

Widaman, K. F., Helm, J. L., Castro-Schilo, L., Pluess, M., Stallings, M. C., & Belsky, J. (2012). Distinguishing ordinal and disordinal interactions. *Psychological Methods, 17,* 615-622.

Wüst, S., Entringer, S., Federenko, I. S., Schlotz, W., & Hellhammer, D. H. (2005). Birth weight is associated with salivary cortisol responses to psychosocial stress in adult life. *Psychoneuroendocrinology, 30,* 591-598.

Zuckerman, M. (1999). *Vulnerability to psychopathology: A biosocial model.* Washington, DC: American Psychological Association.