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Timing matters: Long term effects of adversities from prenatal period up to adolescence on adolescents' cortisol stress response. The TRAILS study

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Summary
Objective: Altered cortisol response is a vulnerability marker for a variety of stress-related diseases and psychiatric disorders. Childhood adversity has been shown to modify this response, but evidence is inconsistent. Effects may differ depending on the timing of exposure, or due to the interplay between pre/postnatal adversity and later adversities. The present study examined the influence of adversity during different timeframes (pre/postnatal, ages 0—5, 6—11, 12—13, 14—15 years), and the interaction between pre/postnatal and later adversity on adolescents' cortisol stress response.
Method: Four salivary cortisol samples were collected before and after a social stress test in 471 16-year-old adolescents from the longitudinal study TRAILS. Data on pre/postnatal exposure to adversities were obtained from Preventive Child Healthcare records and parental reports, subsequent adversities from parental and self-reports.
Results: Pre/postnatal adversity was associated with increased cortisol reactivity. Adversities during ages 0—5 were not associated with cortisol outcomes. Adversities during ages 6—11

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were associated with a high cortisol level, especially in those exposed to pre/postnatal adversity, while adversities during ages 12—13 and 14—15 were associated with a low cortisol level.

Conclusions: Results highlight the importance to take the timing of stress exposure into account. In addition to programming effects, pre/postnatal adversity interacts with childhood adversity in producing deviant cortisol levels. Puberty may be marked by a transition in how adversities affect the HPA-axis, with cortisol hypersecretion before age 11 and hyposecretion after age 11.

1. Introduction

Early life adversity, such as prenatal maternal alcohol use and stressful life events during childhood, has been shown to cause lifelong alterations of neuro-endocrine, behavioral and cognitive functions (Van den Bergh et al., 2008; Weaver, 2009), and increase the risk for developing a wide range of psychiatric, cardiovascular and metabolic disorders in later life (Schlotz and Phillips, 2009). One of the major stress systems associated with early life adversity is the hypothalamic—pituitary—adrenal (HPA) axis, with cortisol (in humans) as its major end product. Substantial evidence shows altered HPA-axis reactivity after early life stress, but with considerable variability in the degree and direction of HPA functioning (Carpenter et al., 2007; Elzinga et al., 2008; Entringer et al., 2009; Luecken et al., 2009; MacMillan et al., 2009; Pesonen et al., 2010). Age of stress exposure and stress sensitization by adversity during the pre- and postnatal period could both contribute to these inconsistencies, and are therefore the subject of our study.

Pre- and postnatal adversities such as prenatal maternal psychosocial stress (Entringer et al., 2009), alcohol use (Hellemans, 2010), and low birth weight (Wüst et al., 2005) have consistently been associated with HPA hyperreactivity to stress, a process known as 'prenatal programming' (Lupien et al., 2009). In contrast, stressful live events during childhood and adolescence has been found to either increase (Pesonen et al., 2010) or decrease (Carpenter et al., 2007; Elzinga et al., 2008; Luecken et al., 2009; MacMillan et al., 2009) HPA responses to psychosocial stressors later in life. The brain regions involved in HPA-axis regulation, such as the hippocampus, frontal cortex and amygdala, start to develop prenatally (Lupien et al., 2009), and are extremely sensitive to stress during the pre/postnatal period. During childhood and adolescence, the involved brain structures have different maturation pathways, and as a result, each structure has specific sensitive periods for exposure to stress (Lupien et al., 2009). In line with this, a cross-sectional study found that sexual abuse during ages 3—5 or 11—13 was associated with reduced hippocampal volume, and abuse during ages 14—16 with reduced frontal cortex volume (Andersen et al., 2008). However, most studies examining effects of stressful live events on cortisol responses have disregarded the timing of trauma (Carpenter et al., 2007; Elzinga et al., 2008; Luecken et al., 2009; MacMillan et al., 2009). To our knowledge, only two studies investigated the specific age at which the adversity occurred (Gunnar et al., 2009a; Pesonen et al., 2010). For example, Pesonen and colleagues showed that separation from parents during childhood was associated with an increased cortisol stress response in adulthood, particularly if the separation occurred between ages 2—7 (Pesonen et al., 2010). In sum, diverging effects of childhood stress on cortisol responses may be due to the age of exposure to the adversity.

A second potential influence of adversity on the HPA-axis regards stress sensitization, whereby pre/postnatal adversities may enhance the probability of stress-induced alterations of the HPA-axis after exposure to subsequent stress. Essex (2002) found that the combination of stress exposure during infancy and preschool years predicted higher daytime cortisol levels in children (age 4.5 years). Furthermore, Rao (2008) found that the combination of early childhood and recent stress predicted the highest cortisol stress response in depressed adults. Whether pre/postnatal adversity modifies the impact of later stressors on HPA-axis functioning has not been addressed in the literature so far, but recent studies have found sensitization effects due to prenatal adversity with respect to depression (Costello et al., 2007) and overall wellbeing (Nomura and Chemtob, 2007). For example, a study on the effects of low birth weight on adolescent depression found that low birth weight did not predict depression in itself, but increased the sensitivity to the depressogenic effects of subsequent stressors in girls (Costello et al., 2007).

Compared to childhood and adulthood, research on HPA function in adolescence is scarce (McCormick et al., 2010). This study extends previous findings by exploring the effects of adversities from the prenatal period up to adolescence on adolescents' cortisol stress responses. We examined adversities during five age periods (pre/postnatal, 0—5 years, 6—11 years, 12—13 years, 14—15 years), to investigate their specific effects on the HPA-axis. We anticipated that effects of adversities on the cortisol responses would depend on their timing, and that pre/postnatal adversity would render individuals more sensitive to persistent effects of later adversities on the HPA-axis.

2. Methods

2.1. Study population

The data were collected in a focus sample of TRAILS (TRacking Adolescents' Individual Lives Survey), a large prospective population study of Dutch adolescents from age 11 to 21. Thus far, three data waves have been completed: T1 (2001—2002, $N = 2230$, age 10—12, 51.0% girls, response rate 76.0%), T2 (2003—2004, $N = 2149$, age 12—15, 51.0% girls, response

rate 96.4%), and T3 (2005–2007, $N = 1816$, age 14–18, 53.0% girls, response rate 81.4%).

During T3, a focus sample from the total TRAILS sample was invited to an experimental session, of whom 715 (96.1%) agreed to do so. Adolescents with an increased risk of mental health problems (lifetime parental psychopathology, living in a single-parent family, or difficult temperament) had a greater chance of being invited. In total, 66.2% of the focus sample were randomly selected from the high-risk TRAILS participants and had at least one risk factor; the remaining 33.8% were randomly selected from the low-risk TRAILS participants. The focus sample represented the whole range of problems seen in a normal population of adolescents. Complete HPA-axis and adversity measurements were available for 640 participants. Adolescents were excluded if stressful life events were assessed more than 3 months before ($n = 60$) or more than six months after the experiment ($n = 60$). In addition, we excluded adolescents with cortisol outliers (>3 SD, $n = 11$), as well as adolescents who used corticosteroids ($n = 38$), leaving a total of 471 (32.3% low-risk) participants for analysis. Included and excluded adolescents did not differ with regard to sex, family composition, parental psychopathology, household income and smoking status, but included adolescents were slightly older (mean difference = two months, $t = -3.79$, $p < .0001$) and lower BMI ($t = 2.61$, $p = .009$). The study protocol was approved by the Central Committee on Research Involving Human Participants (CCMO). Participants provided written informed consent and received a voucher of 30 euro.

2.2. The social stress test

The experimental session during T3 consisted of a number of different challenges (orthostatic stress, spatial-orienting task, gambling task, startle-reflex task, and social stress test). The session started between 0800 h and 0900 h (42.6%) or between 1230 h and 1400 h, and were preceded and followed by a 40-min period of rest. Total duration was about 2 h. Participants filled out a number of questionnaires at the start and end of the session. Participants were asked to refrain from heavy physical activities the day prior to the experimental session and not to smoke or use coffee, milk, chocolate, and other sugar-containing foods during the 2 h before the session. The session took place in sound-proof rooms with blinded windows at selected locations in the participants' residence towns.

The social stress test (Groninger Social Stress Test) involved a standardized protocol inspired by the Trier Social Stress Test (Kirschbaum et al., 1993). Participants were instructed to prepare a 6 min speech about themselves and their lives and deliver this speech in front of a video-camera. They were told that their videotaped performance would be judged by a panel of peers after the experiment. Participants had to speak for 6 min continuously, while the test-assistant watched the performance critically without showing any empathy or encouragement, followed by a 3-min silent interlude. After that, participants had to subtract the number 17 repeatedly, starting with 13,278, while the test-assistant gave negative, frustrating feedback. This mental arithmetic task lasted for 6 min, again followed by 3-min period of silence, after which the participants were thoroughly debriefed about the experiment.

2.3. Measures

2.3.1. Salivary cortisol collection

HPA-axis responses towards the social stress test were assessed by four cortisol samples, before the introduction of the social stress test ('pretest'), directly after the test ('during test'), 20 min after the test ('end of test'), and 40 min after the test ('post test'). Considering the normal delay in peak cortisol responses to experimental stressors (20–25 min) (Kudielka and Wüst, 2010), all samples reflect responses about 20 min earlier. Saliva cortisol was assessed by the Salivette sampling device (Sarstedt, Numbrich, Germany) and stored at −20 °C until analyses. Cortisol was measured directly in duplicate in 100 μl saliva using an in-house radioimmunoassay (RIA) applying a polyclonal rabbit cortisol antibody and 1, 2, 6, 7 3H cortisol (Amersham) as tracer. After incubation for 30 min at 60 °C, the bound and free fractions were separated using activated charcoal. Intra- and inter-assay coefficients of variation for the assays were 5.6–12.6%.

2.3.2. Pre/postnatal adversity

At T1, well-trained interviewers visited the parents at their homes to administer an interview covering prenatal and postnatal events. Birth weight and gestational age, as provided by the obstetrician or midwife, were obtained from files from the Preventive Child Healthcare (PCH) (Reijneveld et al., 2004). Missing PCH data ($n = 82$) were imputed with data obtained from the parents, which correlated highly ($r > .87$) with the PCH data. The measure of pre/postnatal adversity was constructed as the sum score of maternal alcohol use (18.6%), maternal smoking (29.1%), maternal psychological problems during pregnancy (3.6%) or the three months after delivery (3.4%), preterm delivery (≤33 weeks; 1.1%), low birth weight (≤2500 g; 6.8%), and hospitalization of mother or child within one month after delivery (mother 13.0%, child 24.4%). In total, 35.5% of the participants were not exposed to any, 42.7% to one, 14.9% to two, 4.7% to three, 1.7% to four, 0.4% to five, and 0.2% to six pre/postnatal adversities.

2.3.3. Adversities during ages 0–5 (early childhood) and 6–11 (middle childhood)

At T1, information on major stressful events (hospitalization, parental divorce, death of family member, out-of-home placement, parental addiction, and parental mental health problems) and age of occurrence was obtained from the parental interview. Sum scores were made of the events during ages 0–5 and ages 6–11. At T2, both parents and adolescents were asked to rate the overall stressfulness of these two periods (age 0–5 and 6–11), on a scale ranging from 1 (not at all stressful) to 10 (extremely stressful). The mean of the standardized parent and adolescent reports was used as an overall index of experienced stress.

2.3.4. Adversities during ages 12–13 (early adolescence)

At T2, adversities in the past two years were assessed by means of a self-report questionnaire. The total number reflected the sum of 25 adversities such as death/sickness

of family member, parental divorce, conflicts, and bullying. Furthermore, both parents and adolescents were asked to rate the overall stressfulness of the last two years on a scale ranging from 1 (not at all stressful) to 10 (extremely stressful). The mean of the standardized parent and adolescent reports was used as an overall index of experienced stress.

2.3.5. Adversities during ages 14—15 (middle adolescence)

At T3, information on adversities in the past two years was obtained from the adolescents during a semi-structured interview covering a wide range of stressful experiences. These events included conflicts, loneliness, physical or sexual assault/abuse, bullying/gossiping, lack or loss of friends, psychological/addiction problems of family or friends, out-of-home placement, running away from home, death/sickness of family member, hospitalization, and parental divorce. The number of events reflected the sum of adversities that has occurred. Chronic stress reflected the summed duration of the following adversities: physical or sexual abuse/assault, bullying/gossiping, lack of friends, conflicts, severe problems of family members or friends, out-of-home placement, and running away from home. Duration of the adversities was measured in months.

2.3.6. Covariates

The following variables were included as covariates: sex, use of oral contraceptives (OC), timing of the experiment, current depressed mood, social economic status (SES), body mass index (BMI), and smoking (yes/no) (Kudielka and Wüst, 2010). Current use of OC was assessed at the start of the session. Current depressed mood was assessed at the start of the session, with the Dutch version of the short Profile of Mood Scale (POMS) (Wald and Mellenbergh, 1990) that includes eight items (down, helpless, sad, lonely, unhappy, unworthy, melancholic, desperate) rated on a five-point scale (1 = not at all, 2 = a little, 3 = partly, 4 = kind of, 5 = very much; Cronbach's alpha = .87). SES was based on parental education and occupation, and household income (Amone-P'Olak et al., 2009). BMI (weight in kg/height in m^2, measured by test-assistants) and smoking were assessed as part of the regular T3 measurements, on average 0.59 months (SD = 2.91) after the experimental session.

2.4. Statistical analyses

Descriptive statistics were calculated and associations among adversity variables were examined by correlation coefficients. Repeated-measures general linear models (GLM, Greenhouse—Geisser corrected) were used to examine (1) the main effects of adversities per period and (2) the interaction of pre/postnatal risk with each later adversities on the HPA-axis. Adversity variables were tested simultaneously and hence their effects were adjusted for each other. Investigated outcome variables were cortisol reactivity and cortisol overall level. Cortisol reactivity reflects the pattern of response, while cortisol overall level represents the average level across all four samples. Cortisol data were log-transformed to attain normality. Adversity variables were transformed to z-scores. Analyses were conducted with SPSS 16 and adjusted for sex, OC use, timing of the experiment, current depressed mood, SES, BMI and smoking (Kudielka and Wüst, 2010). Bonferonni corrected post hoc analyses were conducted to examine whether effects of adversities on the HPA-axis were moderated by sex (Elzinga et al., 2008; Pesonen et al., 2010) or OC use (Viau, 2002). To illustrate the effects of adversities, we divided the sample for each adversity based on: low (none), intermediate (<1 SD), and high (>1 SD) scores. Significant effects were assumed at $\alpha < .05$.

3. Results

3.1. Demographic and adversity characteristics

Descriptive statistics can be found in Tables 1 and 2. As might be expected, the correlations between the adversity measures tended to drop with increasing time between the periods.

3.2. Main effects of adversities

The social stress test elicited significant cortisol responses ($F_{3,1323} = 11.84$, $p < .0001$; quadratic effect $F_{1,441} = 28.02$, $p < .0001$), which was associated with pre/postnatal adversities (Table 3). Overall level of cortisol was associated with the number of adversities during late childhood (ages 6—11), early adolescence (ages 12—13), and middle adolescence (ages 14—15) (Table 3). As shown in Fig. 1, pre/postnatal adversities were associated with a high cortisol stress

Table 1 Demographic and adversity characteristics of the study sample (N = 471).

	Mean (SD) or percentage
Demographics	
Boys/FC-girls/OC-users	48.0%/35.9%/16.1%
Age (years)	15.54 (0.60)
BMI (kg/m^2)	21.02 (2.91)
Smokers	21.8%
SES	0.11 (0.76)
Current depressed mood	9.16 (8.13)
Adversities	
Pre/postnatal adversities	−0.06 (0.94)
Age 0—5	
Number	0.17 (1.09)
Overall	−0.04 (0.89)
Age 6—11	
Number	0.06 (1.00)
Overall	−0.05 (0.89)
Age 12—13	
Number	−0.08 (0.88)
Overall	−0.08 (0.85)
Age 14—15	
Number	0.51 (1.21)
Chronic	0.45 (1.36)

Note: adversity variables were standardized to mean 0 and standard deviation 1 to ease comparison. FC-girls, free-cycling girls; OC-users, oral contraceptive users; BMI, body mass index; SES, social economic status; number, number of adversities; overall, overall stress; chronic, duration of adversities.

Table 2 Bivariate associations between the adversities used in this study.

	Pre/postnatal	Age 0–5		Age 6–11		Age 12–13		Age 14–15
	Number	Number	Overall	Number	Overall	Number	Overall	Number
Age 0–5								
Number	0.24***							
Overall	0.04	0.28***						
Age 6–11								
Number	0.20***	0.40***	0.14**					
Overall	0.08	0.14**	0.42***	0.15***				
Age 12–13								
Number	0.10*	0.13**	0.15**	0.09	0.21***			
Overall	0.01	0.02	0.27***	0.02	0.48***	0.32***		
Age 14–15								
Number	0.08	0.08	0.21***	0.05	0.18***	0.33***	0.21***	
Chronic	0.11*	0.15**	0.20***	0.12**	0.13**	0.23***	0.15**	0.75***

Note: pre-postnatal, pre/postnatal adversity; age 0–5, adversity during age 0–5; age 6–11, adversity during age 6–11; age 12–13, adversity during age 12–13; age 14–15, adversity during the two years preceding the social stress test; number, number of adversities; overall, overall stress; chronic, duration of adversities.

* $p < .05$.

** $p < .01$.

*** $p < .001$.

response (Fig. 1a). Adversities during late childhood were related to a high overall level of cortisol (Fig. 1b), while adversities during early adolescence (Fig. 1c) and chronic stress during middle adolescence (Fig. 1d) were related to a low overall level of cortisol. The effects were not modified by sex or OC-use.

3.3. Interaction of pre/postnatal adversity and adversities during other developmental periods

Cortisol reactivity was not associated with the interaction of pre/postnatal adversities and any of the subsequent adversity measurements. Overall level of cortisol was associated

Table 3 General linear model examining effects of adversities on cortisol reactivity and overall level during the social stress test.

	Cortisol reactivity		Cortisol overall level	
	F (*df*)	*p*	*F* (*df*)	*p*
Demographics				
Boys/FC-girls/OC-users	9.13 (*6,1323*)	<.0001	7.19 (*2,441*)	.001
BMI (kg/m^2)	1.71 (*3,1323*)	.18	1.30 (*1,441*)	.25
Smoking status	6.14 (*3,1323*)	.002	1.37 (*1,441*)	.24
SES	1.37 (*3,1323*)	.26	0.56 (*2,441*)	.45
Current depressed mood	0.19 (*3,1323*)	.82	0.11 (*1,441*)	. 74
Session time	2.77 (*3,1323*)	.06	54.92 (*1,441*)	<.0001
Adversities				
Pre/postnatal	5.17 (*3,1323*)	.006	0.37 (*1,441*)	.54
Age 0–5				
Number	0.03 (*3,1323*)	.97	0.22 (*1,441*)	.64
Overall	0.08 (*3,1323*)	.92	0.08 (*1,441*)	.77
Age 6–11				
Number	0.65 (*3,1323*)	.52	8.08 (*1,441*)	.005
Overall	2.07 (*3,1323*)	.13	1.93 (*1,441*)	.17
Age 12–13				
Number	1.21 (*3,1323*)	.30	4.80 (*1,441*)	.03
Overall	1.20 (*3,1323*)	.30	0.01 (*1,441*)	.93
Age 14–15				
Number	0.11 (*3,1323*)	.90	1.79 (*1,441*)	.18
Chronic	0.78 (*3,1323*)	.46	5.85 (*1,441*)	.02

Note: FC-girls, free-cycling girls; OC-users, oral contraceptive users; BMI, body mass index; SES, social economic status; pre/postnatal, pre- and postnatal adversity; age 0–5, adversity during ages 0–5; age 6–11, adversity during ages 6–11; age 12–13, adversity during ages 12–13; age 14–15, adversity during the two years preceding the social stress test; number, number of adversities; overall, overall stress level; chronic, summed duration of adversities.

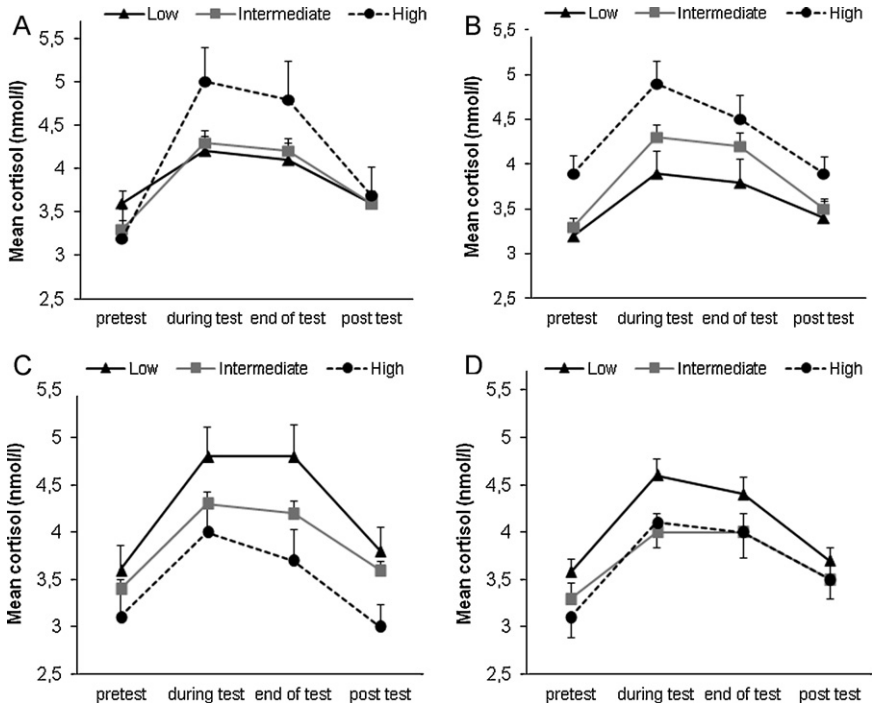

Figure 1 (a—d) Illustrations of the effects of adversities on adolescents' cortisol responses to social stress. (a) The graphical presentation of the cortisol response to social stress with respect to pre/postnatal adversity. Subjects with the highest number of pre/postnatal adversities had the highest cortisol reactivity ($F_{3,1323} = 5.17$, $p = .006$; quadratic effect $F_{1,441} = 8.98$, $p = .003$). The significant quadratic effect indicate a significant increase of cortisol compared to pre- and post levels. (b) Subjects with the highest number of late childhood adversities (ages 6—11) displayed the highest cortisol overall level (mean level of cortisol across the four cortisol measurements, $F_{1,441} = 8.08$, $p = .005$). (c) Subjects with the highest number of adversities during early adolescence (ages 12—13) had the lowest cortisol overall level ($F_{1,441} = 4.80$, $p = .03$). (d) Subjects with a high amount of chronic stress during middle adolescence (ages 14—15) had the lowest cortisol overall level ($F_{1,441} = 5.85$, $p = .02$). Error bars indicate standard errors of the mean.

with the interaction of pre/postnatal adversities and number of adversities during late childhood ($F_{1,440} = 4.35$, $p = .04$). As Fig. 2 shows, the combination of many adversities during both periods was associated with a high overall level of cortisol. Sex and OC-use had no modifying effects.

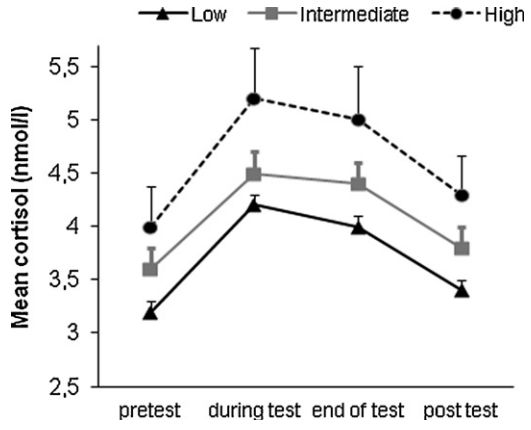

Figure 2 Illustrations of the interaction effect of pre/postnatal by late childhood adversity on adolescents' cortisol responses to social stress. Fig. 2 shows the interaction between perinatal adversity and late childhood adversity (ages 6—11) on cortisol overall level, whereby subjects with a combination of both types of adversity had a high cortisol overall level ($F_{1,440} = 4.35$, $p = .04$). Error bars indicate standard errors of the mean.

4. Discussion

This study highlights the importance of pre- and postnatal adversity in the long-term programming of HPA-axis regulation and sensitization to subsequent life stressors. The transition in cortisol activity from hypersecretion after adversities before age 11 and hyposecretion afterwards emphasizes puberty as a major developmental period of the HPA-axis. Our findings add to the accumulating evidence that adolescents are particularly sensitive to stressful experiences and demonstrate the need to focus on the timing of stress.

These findings are important for several reasons. First, this study shows that pre/postnatal adversities are related to adolescents' stress response, independent of later adversities in life, and as such underscores the importance of early programming of the HPA-axis for cortisol responses later in life. This effect is probably due to a reduced feedback mechanism caused by a decrease of hippocampal glucocorticoid and mineralcorticoid receptors (see for review Weinstock, 2005). Several studies have reported associations between pre/postnatal adversities and HPA-axis hyperactivity during stress tasks in infancy (Oberlander et al., 2008) and adulthood (Wüst et al., 2005; Entringer et al., 2009), but they did not take into account that some adversities may not be restricted to the pre/postnatal period but continue throughout childhood and adolescence. By controlling for adversities later in life, we showed that the increased cortisol response

after exposure to pre/postnatal adversities probably actually reflect long-lasting effects of these adversities, and not of adversity later in life.

Second, adversities during late childhood were associated with a high mean level of cortisol (hypersecretion), while adversities during early and middle adolescence were associated with a low mean level of cortisol (hyposecretion). As opposed to cortisol reactivity measures, the mean level of cortisol reflects basal activity levels rather than stress responsiveness of the HPA-axis. The high cortisol level after adversity during late childhood may be due to a combination of incomplete maturation of the negative feedback mechanism of the HPA-axis in prepuberty (Romeo, 2010a) and upregulated corticotropin-releasing hormone (CRH) receptors in the amygdala (Tottenham and Sheridan, 2009). The low level after adversity during early and middle adolescence, may be explained by downregulation of receptors at different levels of the HPA-axis, increased feedback sensitivity, and morphological changes (Heim et al., 2000b), which may not be permanent (Lupien et al., 2009). The frontal cortex is most likely to be affected by stress in this period: a smaller frontal cortex volume has been reported to be associated with abuse during middle adolescence (Andersen et al., 2008).

The change from hyper- to hyposecretion of the HPA-axis due to adversity was found to occur around the start of puberty, at about age 11. This highlights the importance of timing of stress exposure during the development of the brain regions involved in regulating the HPA-axis (Lupien et al., 2009). The change in direction during early adolescence is consistent with findings in dysphoric youth that also revealed a developmental switch during puberty (Hankin et al., 2010). In this study, prepubertal dysphoric children showed a low cortisol stress response, while postpubertal dysphoric adolescents displayed a high response to the stressor. The difference in direction of the switch between this study (hypo to hyper) and our own (hyper to hypo) addresses the need to study the dynamic change of HPA-axis functioning over time with regard to adversity and concurrent psychopathology. For example, the longitudinal study of Trickett et al. (2010) found that victims of sexual abuse initially show cortisol hypersecretion that changes over time into hyposecretion.

Third, we found no effects of childhood adversity before age 5 on cortisol in our sample, which contrasts findings in an elderly sample, in which individuals who were separated from their parents had increased cortisol responses to stress, especially those who experienced the separation during ages 2—7 (Pesonen et al., 2010). However, results in adults and adolescents are not directly comparable, since deficits in HPA-axis related brain structures may not become apparent until in late adulthood when structures have matured (Isgor et al., 2004) or change dynamically over time (Trickett et al., 2010). Furthermore, cortisol responses to stress changes over time during the development, with children's reactions to stress marked by cortisol hypo-responsiveness during preschool years (Gunnar and Quevedo, 2007) and by hyperresponsiveness during puberty (Gunnar et al., 2009b; Romeo, 2010b) compared to adults. Furthermore, we found no effect of adversities during childhood or adolescence on cortisol reactivity in our sample, which contrasts findings from other studies conducted in adolescents (MacMillan et al., 2009) and adults (Carpenter et al., 2007; Elzinga et al., 2008; Luecken et al., 2009) reporting blunted cortisol reactivity after childhood stress. However, these studies did not specifically examine timing of childhood adversity. Concurrent psychopathology may also contribute to the divergent findings in literature, as is illustrated by several studies reporting only associations of childhood adversity with altered HPA-axis functioning in participants with current depression or unresolved trauma (Heim et al., 2000a; Pierrehumbert et al., 2009).

Fourth, the significant interaction of pre/postnatal adversities with childhood stress demonstrates, for the first time in humans, stress-sensitization effects of pre/postnatal adversities. In other words, apart from direct programming effects, pre/postnatal adversities also render individuals more sensitive to develop persistent alterations in HPA-axis functioning following adversity during late childhood. Previous studies have shown a synergistic effect of low birth weight (Nomura and Chemtob, 2007) with childhood abuse in increasing the risk for psychiatric problems, possibly due to HPA-axis alterations. Only one study, to our knowledge, has examined the possible mediating role of the HPA-axis, with daytime cortisol profile as a measure of HPA-axis functioning. This study confirmed involvement of the HPA-axis in the relationship between prenatal maternal anxiety and depressive symptoms in adolescence (Van den Bergh et al., 2008).

Our study had several limitations. First, the retrospectively collected adversities may have underestimated the incidences of adversity (Hardt and Rutter, 2004); especially the adolescents recall perceptions of stress during ages 0—5 is likely to be biased. However, the use of multiple informants reduced the bias associated with mono-informant and self-report information. Second, the specific age periods were driven by the design of the TRAILS study, preventing investigation of other or smaller periods. In addition, we measured different events per time period and we did not measured overall stress and chronic stress in all periods and had no parental data on stress during ages 14—15, which calls for caution until the finding of age-dependent relationships is replicated in other studies. Third, Gustafsson et al. (2010) examined the association of moderate and high amounts of adversity on day time cortisol levels in children, and found an inverse U-pattern of association, although post hoc analyses did not yield any curvilinear effects of our adversity measurements on cortisol. Fourth, we did not assess ACTH data, which would have allowed for a more complete description of underlying mechanisms in the relation between adversity and the HPA-axis. Fifth, the design of the study did not establish causal relationships. Despite these limitations, this study provides a starting point for future research of the importance of timing of adversity on HPA-axis development and functioning.

5. Implications and future research

Both hypo- and hyper-responsiveness to stress have been associated with vulnerability to a variety of medical and mental diseases (e.g. Erickson et al., 2003). Early adversities have been shown to have epigenetic effects on gene expression (e.g. Oberlander et al., 2008; Weaver, 2009), which can explain how the early environment affects the adult phenotype. Since genetic influences operate throughout life (Schlotz and Phillips, 2009), studies disentangling genetic

and fetal environmental factors on stress responses and (mental) health should investigate their interplay across development.

6. Conclusions

This study provides evidence that there are particular stages of development (sensitive periods) when adversity exerts an increased effect on the developing HPA-axis. Based on these findings, there may be different adversity related syndromes associated with particular ages of adversities and also with specific underlying regional brain changes. Future studies should explore potential reversibility of the HPA-axis stress vulnerability, especially early in life, to lower the risk for future stress-related diseases and psychiatric disorders.

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Conflicts of interest statement

All authors declare that they have no conflicts of interest.

Contributors

All authors contributed to and have approved the final manuscript.

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