The sensitive sex
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2010

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Parental depressive symptoms and adolescents’ cortisol responses to social stress.

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What is inherited is the manner of response to a given environment.
ABSTRACT
Depression runs in families and is considered a stress-related disorder. Familial risk for depression may be transmitted via deviant psychophysiological stress responses from parent to child. In this study, we examined the association between parental depressive symptoms (PDS) and adolescents’ cortisol responses to an acute social stress test. Data were collected as part of the third assessment wave of TRAILS (TRacking Adolescents’ Individual Lives Survey), a large prospective population study of Dutch adolescents, and concern 346 adolescents (age 15-17 years, 42.2% female) who participated in a laboratory session including a social stress test (public speaking and mental arithmetic). Four saliva cortisol samples were collected before, during and after the social stress test. Lifetime PDS were assessed by self-reports obtained from both biological parents. Adolescents’ current depressed mood was included as covariate in the analyses. In case of an effect of PDS on the cortisol response, we tested if this effect was mediated by experienced life stress. PDS was associated with daughters’ cortisol responses (B = -52.32, p = .02), but no association was found in sons (B = -5.44, p = .77). Girls whose parents experienced depressive problems displayed blunted cortisol responses compared to girls whose parents did not have such problems. The effect of PDS on the cortisol response in girls was not mediated by experienced life stress (S = 0.01, p = .99). Concluding, PDS are associated with blunted cortisol responses to acute social stress in daughters, but not in sons. The prognostic value of blunted cortisol responses in the development of depressive symptoms in adolescents at risk for familial depression needs further attention, preferably in longitudinal study designs.
INTRODUCTION

Depression is generally considered a stress-related disorder (Brown and Harris, 1989; Kessler 1997; Tennant 2002). It runs in families, and having a parent with a history of depression is a strong predictor for depressive symptoms in offspring (Pilowsky et al., 2006; Schreier et al., 2006). From twin, adoption and family studies we know that depression is moderately heritable (e.g. Sullivan et al., 2000). The genetic transmission of risk for depression may run partly through altered functioning of the physiological stress system. Support for this notion comes from several studies that suggest a genetic influence on the relationship between stress and depression (Kendler et al., 1995; Caspi et al., 2003; Gotlib et al., 2008). Consistent with this, we showed that adolescents with depressed parents are more prone to develop depressive symptoms after stress than adolescents without depressed parents (Bouma et al., 2008). The hypothalamic-pituitary-adrenal (HPA) axis is a logical candidate for the link between stress experience and depression (Holsboer et al., 1995; Plotsky et al., 1998; Kendler et al., 1999). Altered HPA axis responses to stress have been found in patients suffering from depression, but also in their healthy family members (e.g. Holsboer et al., 1995).

In humans, actual and perceived stressors evoke a rapid response of the sympathetic nervous system (SNS) and a slower, longer response mediated by the HPA axis (Sapolsky et al., 1986). The activation of the central nervous system causes the release of corticotropin releasing hormone from the hypothalamus, adrenal corticotrophic hormone from the anterior pituitary, and cortisol from the adrenal cortex. Among other functions, cortisol converts fat into glucose and inhibits the immune system. Ideally, elevated cortisol levels function as a suppressive mechanism dampening the initial response to re-establish homeostasis via negative feedback mechanisms in the hippocampus (Jacobson and Sapolsky, 1991). Cortisol release during stress is essential for survival, but prolonged HPA axis activation may lead to deregulation of the system, resulting in hyper- or hypo-cortisol secretion to subsequent stressors (McCleery and Harvey, 2004).

Although there is much evidence that children of depressed parents have behavioral and emotional problems (e.g. Cicchetti and Toth, 1998), the association between PDS and offspring physiological responses to acute stress are less well-known. Studying responses to stress is necessary to understand how depression is transmitted across generations. In this study, we tested if familial risk for depression, operationalised as lifetime PDS, influenced adolescent offspring’s cortisol responses to a standardised acute social stress test.
Gender is an important determinant of both vulnerability to depression and physiological responses to stress. Twice as many women as men suffer from depression (Nolen-Hoeksema, 2001), a gender difference emerging in adolescence (Angold et al., 1998; Hankin et al., 1998). This might be due to dissimilar increases in sex hormones in adolescent boys and girls, which can modulate the activation and feedback mechanisms of the HPA axis in diverging manners (McCormick and Matthews, 2007). We showed that cortisol responses to stress differed indeed between adolescent boys and girls (Bouma et al., 2009). This is consistent with findings from Wang and co-workers (2007), who showed that neural responses to psychological stress were different in men and women. Because of dissimilarities in vulnerability and stress responses, we examined the effect of PDS on the cortisol response to social stress separately for boys and girls. Our main hypothesis was that genetic risk for depression, operationalised by presence or absence of PDS, was associated with deviant cortisol responses to an acute social stress test in adolescent boys and girls. Since deregulation of the HPA axis can result in both hyper- and hyposecretion of cortisol, we had no clear expectations regarding the direction of the effects.

Familial risk for depression and deviant responses to stress are not solemnly transmitted by genes, but also by social and behavioural factors. PDS can lead to a stressful or non-supportive family environment (e.g. Goodman and Gotlib, 1999; Jaser et al., 2005), and hence be associated with over- or under activation of the HPA axis through gene-environment correlations (Lupien et al., 2009). To explore this pathway, we examined to what extent associations between PDS and offspring stress responses (if any) were due to life stress experienced by the adolescents.

**METHODS**

**Sample and Setting**

Data were collected in a focus sample of TRAILS (TRacking Adolescents’ Individual Lives Survey), a large prospective population study of Dutch adolescents with bi- or triennial measurements from age 11 to at least age 25. For a detailed description of this cohort please see Huisman et al. (2008). At the third assessment wave (T3), a group of adolescents were invited to perform a series of laboratory tasks (hereafter referred to as the experimental session), among which was the Groningen Social Stress Test (GSST). Before and after this test, four salivary cortisol samples were taken. In total, 715 adolescents participated in the experiments. Information about lifetime depressive symptoms of both (biological) parents was available for 407 of the participants.
To increase the power to detect mental health-related differences in response patterns, adolescents with an increased risk for mental health problems had a greater chance of being selected for the experimental session. Increased risk was defined based on temperament (high frustration and fearfulness, low effortful control), parental psychopathology (depression, anxiety, addiction, psychoses, or antisocial behaviour), and environmental risk (living in a single-parent family). Of the 407 adolescents participating in the present study, 59.5% had at least one of the above-described risk factors. Lifetime parental depressive symptoms were obviously associated with the selection factor parental psychopathology, but not with temperament or single parenthood. Please note that, although high-risk adolescents were oversampled, the sample included the total range of mental health problems present in a community population of adolescents, only in a different distribution. Preliminary analyses showed that risk status (high or low) was not associated with the cortisol response to social stress. Detailed information on the response rates within each stratum can be obtained from the corresponding author. Experimental sessions took place on weekdays, in soundproof rooms with blinded windows at selected locations in the participants’ residence town; lasted about three hours and 15 minutes; and started between 08:00h and 09:30h or between 12:30h and 02:30h (59%). Although free salivary cortisol levels are higher in the morning due to the circadian rhythm of cortisol production, morning and afternoon cortisol responses to social stress were expected (Kudielka et al., 2004) and found to be comparable in this sample (Bouma et al., 2009). We asked the participants to refrain from smoking and from using coffee, milk, chocolate, or other sugar-containing foods in the two hours before the session, since these substances are known to influence cortisol levels. At the start of the session, the test assistant, blind to the participants’ risk status, explained the procedure and administered a short checklist on current medication use, menstrual cycle, quality of sleep, and physical activity in the last 24 hours. The experimental protocol was approved by the Central Committee on Research Involving Human subjects (CCMO).

Of the 407 adolescents from whom we had information of both biological parents, girls using OC (n = 58) were excluded from the analyses since an earlier study of this sample indicated that OC-users displayed no cortisol response towards the social stress test at all (Bouma et al., 2009). Additionally, we discarded one adolescent because of use of corticosteroid-containing medication, while two adolescents were excluded because of two or more missing cortisol variables. Hence, 346 adolescents (42.2% girls, mean age 15.99, SD = 1.02) were included in the statistical analyses.
The Groningen Social Stress Test
The GSST protocol was inspired by the Trier Social stress test (TSST; Kirschbaum et al., 1993), and described in detail by Bouma et al., (2009). In short, the GSST involves six minutes preparation of speech, followed by seven minutes speaking in front of a camera, then three minutes silence without speech, followed by six minutes of performing difficult mental arithmetic, and another three-minute interlude. The interludes were meant to assess cardiovascular recordings without the disturbance of speech. The participants were debriefed directly after the test. The GSST encompasses the three most important triggers of HPA axis: uncontrollability, threat of failure, and fear of negative social evaluation (Dickerson and Kemeny, 2002), and has shown to elicit significant changes in heart rate and in the HPA-system (Benschop et al., 1998; Van der Pompe et al., 1998; Bouma et al., 2009).

Measures
Cortisol was assessed from saliva by the Salivette sampling device (Sarstedt, Numbrecht, Germany). Salivary cortisol levels rise about 15 to 20 minutes after a psychological stressor (Sharpley and McLean, 1992). Sample Ce2 was taken just before the start of the GSST and reflects HPA axis activity when participants filled out a rating scale while sitting quietly. Ce3 was collected directly after the end of the GSST and reflects response of the HPA axis during speech. Ce3 and Ce4, collected 20 respectively 40 minutes after the end of the GSST, are considered measures of post-stress activity. After the test, salivettes were stored at -20°C until analysis. Details on intra- and inter-assay coefficients of variation can be found in Bouma et al., (2009). For 328 (95.8%) adolescents none of the cortisol samples were missing. In the remaining 4.2% if the sample, one experimental sample was missing due to detection failures in the lab (50%) or insufficient saliva in the tubes (50%). Missing values were imputed on the basis of the group mean and standard deviation of the missing cortisol sample and the mean of participants’ other cortisol samples.

Parental depressive symptoms (PDS) were indicated by self-reports of lifetime depressive symptoms, given by both biological parents at T3. The main DSM-IV criteria for a Major Depressive Episode (i.e., sadness, loss of pleasure/interest, sleep and appetite problems, poor concentration, feelings of guilt, suicidal ideation; minimal duration two weeks) were described, followed by a series of questions to assess lifetime prevalence, treatment and timing of the first and last episode. Preliminary analyses indicated that the timing of the depressive episode(s) was not associated with offspring’s cortisol responses. In our sample, 25.7% of the fathers and 36.7% of the mothers reported the occurrence of one or more lifetime depressive episodes (43.9% only one parent, 9.2% both parents). This relatively
high prevalence compared to other adult population samples in the Netherlands (Bijl and Ravelli, 2000) and Europe (ESEMed, 2004) is due to the oversampling of adolescents with parental psychopathology.

*Experienced life stress* was assessed at T2 and T3 by a parent questionnaire developed by TRAILS. Parents could rate the stressfulness of their child’s life from age 0 until age 5, age 6 until age 11, age 11 until age 13 at T2 and from age 13 until age 16 at T3, on a ten-point scale ranging from 1 (totally not stressful) to 10 (very stressful). Ratings were missing for 15 adolescents. A total score for experienced life stress was formed by computing the weighted mean of reports at T2 and T3. This score ranged from 1.06 (5.4%) to 9.00 (0.3%), with a mean of 3.25 (SD = 1.66).

*Adolescents’ depressed mood* was assessed at the start of the experimental session, by the Dutch version of the short Profile of Mood Scale (POMS; Wald and Mellenbergh, 1990). The scale includes eight items describing current mood (down, helpless, sad, lonely, unhappy, unworthy, melancholic, desperate), which could be rated on a five-point scale (1 = not at all, 2 = a little, 3 = partly, 4 = kind of, 5 = very much). Cronbach’s alpha was 0.84. The scale score represents the mean item score.

**Statistical analysis**
Analyses were performed in SPSS (Version 14.0). Differences in means and frequencies between boys and girls were examined by t-tests and $\chi^2$-tests, respectively. Associations between variables were investigated by Pearson’s correlation coefficients. The strength of the cortisol response was indicated by the area under the curve with respect to the increase (AUCi) (after Pruessner et al., 2003), based on the four above-described cortisol samples. The hypothesis that PDS were associated with cortisol responses in offspring was tested by means of linear regression analysis, with the cortisol AUCi as outcome variable and PDS as predictor. Depressed mood can influence the cortisol response to social stress (Burke et al., 2005) and was therefore included as covariate. If PDS were significantly associated with the AUCi, we tested if this effect was mediated by adolescents’ experienced life stress. The mediation pathway was tested by the Sobel test (Sobel, 1982), provided by Kristopher Preacher (www.people.ku.edu/~preacher/sobel/sobel.htm). When the Sobel test was significant, the amount of mediation was estimated by the relative reduction in the regression coefficient of PDS after including the mediator in the model. Analyses were stratified for boys and girls. Data of morning and afternoon sessions were pooled since we previously showed that responses to the GSST were similar in
both morning and afternoon sessions (Bouma et al., 2009). A p-value < .05 was considered statistically significant.

RESULTS

Descriptive statistics
Descriptive statistics are given in Table 1, stratified by gender. Boys and girls differed with respect to age, cortisol levels during the social stress test, general level of experienced life stress, and depressed mood. See Table 2 for bivariate associations between the variables under study.

<table>
<thead>
<tr>
<th>Table 1. Descriptive statistics</th>
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<tbody>
<tr>
<td>Boys</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>Age (in years)</td>
</tr>
<tr>
<td>Ce2 (in nmol/L)</td>
</tr>
<tr>
<td>Ce3 (in nmol/L)</td>
</tr>
<tr>
<td>Ce4 (in nmol/L)</td>
</tr>
<tr>
<td>Ce5 (in nmol/L)</td>
</tr>
<tr>
<td>AUCi (in nmol/L)</td>
</tr>
<tr>
<td>Stress (1-10)</td>
</tr>
<tr>
<td>Depressed mood (1-5)</td>
</tr>
</tbody>
</table>

Note: Ce2 = cortisol before the Groningen Social Stress Test (GSST), Ce3 = cortisol during the GSST, Ce4 = cortisol immediately after the GSST, Ce5 = cortisol 20 minutes after the GSST, AUCi = area under the curve with respect to the increase.

Effect of parental depressive symptoms on offspring cortisol responses
PDS were associated with the cortisol response in daughters, but not in sons (see Table 3). These results are graphically presented in Figure 1 (girls) and Figure 2 (boys). The size of the effect of PDS on daughters’ responses was comparable to a Cohen’s d of 0.4, indicating a medium-sized effect (Cohen 1988). In the total sample, the two-way interaction between gender and PDS showed a marginally significant effect (B = 50.94, p = .08). As could be expected from the lack of association between experienced life stress and cortisol responses, the effect of parental depressive symptoms on the cortisol response in girls was not mediated by adolescent experienced life stress (Sobel test statistic = 0.01, p = .99).
Table 2. Bivariate associations between the variables under study

<table>
<thead>
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<th>1.</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
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<tbody>
<tr>
<td>1. Parental depressive symptoms</td>
<td>.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>2. Experienced life stress</td>
<td>.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>.15</td>
<td>-.03</td>
</tr>
<tr>
<td>3. Adolescent depressed mood</td>
<td>.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.28</td>
<td></td>
<td>-.02</td>
</tr>
<tr>
<td>4. AUC&lt;sub&gt;i&lt;/sub&gt;</td>
<td>-.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-.05</td>
<td>.13</td>
<td></td>
</tr>
</tbody>
</table>

**Bold** = significant association at p < .05. <sup>b</sup> = point biserial correlations.

*Note:* Girls' correlations are printed above the diagonal; boys' correlations below.

Table 3. Effect of parental depressive symptoms on offspring' cortisol response

<table>
<thead>
<tr>
<th></th>
<th>Girls</th>
<th>Boys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>S.E.</td>
</tr>
<tr>
<td>PDS</td>
<td>-52.32</td>
<td>22.67</td>
</tr>
<tr>
<td>ADM</td>
<td>-0.36</td>
<td>9.94</td>
</tr>
</tbody>
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*Note:* PDS = parental depressive symptoms, ADM = adolescent depressed mood.

Figure 1. Mean salivary cortisol responses of girls with (PDS) and without (no PDS) parents with lifetime depressive symptoms.
DISCUSSION

Our objective was to study if familial risk for depression influenced cortisol responses to a standardised social stress test in adolescents. We used lifetime depressive symptoms of both biological parents as a proxy for familial risk. Findings indicated that daughters of parents with a history of depressive symptoms exhibited a blunted cortisol response to the social stress test, while daughters of parents without such history showed the expected curvilinear cortisol response. Sons of parents with a history of depressive symptoms did not differ from sons of parents without a history of depressive symptoms regarding their cortisol response to social stress.

Figure 2. Mean salivary cortisol responses of boys with (PDS) and without (no PDS) parents with lifetime depressive symptoms.
The gender-specific effect of PDS supports the growing body of evidence that women respond differently to stressors than men (Rudolph 2002; Hammen et al., 2004). Although genetic factors are involved in the aetiology of depression, not much evidence is found for a direct effect in the increased risk in women (Kendler and Prescott, 1999). Despite this, genetic factors might indirectly increase this risk by interactions with gonadal hormones, known to influence neurotransmitter functioning and circadian rhythms which can influence coping responses to stress (Parry, 1995; McCormick and Mathews, 2007). Moreover, adolescent girls seem more vulnerable to stress than pre-adolescent girls (Cyranowski et al., 2000; Oldehinkel et al., 2008) and pubertal status have been found to interact with risk for depression (Silberg et al., 1999). Genetic polymorphisms in the mineralocorticoid and glucocorticoid receptor, involved in the HPA axis were differently associated with the cortisol response in men and women (Kumsta et al., 2007) and adolescent boys and girls (Chapter 6).

Blunted cortisol responses can be a result of down-regulation of the HPA axis, which has been suggested to be an adaptive response to expected damaging effects of future stressors (e.g. Fries et al., 2005; Tarullo and Gunnar, 2006). Although it may initially be adaptive, suppressed cortisol activity has been associated with a number of somatic and mental health risks (e.g. Heim et al., 2000; Charmandari et al., 2003), and may hence be a reason for concern. Blunted responses to similar social stress paradigms as the one used in the present study have been found before, and were associated with negative family relationships (Luecken et al., 2009), mild childhood stress (Gunnar et al, 2009), adverse childhood stress (Elzinga et al., 2008), and lifetime stress (Fries et al., 2005). In our study, the effect of PDS on daughters’ cortisol responses was not due to overexposure to stressful situations, at least not as assessed by our parent-report measures. Possibly, the nature of the stressors reflected in this measure was too heterogeneous to have a unidimensional effect on the cortisol response. As Lupien et al. (2009) showed in a recent review, childhood stressors may lead to both over- and under secretion of cortisol, depending on the nature of the stressors. At any case, general childhood stress levels, though associated with PDS, could not explain the association of PDS with cortisol responses to social stress in the present study.

Our findings indicate a different cortisol response to stress in girls at familial risk for depression compared to girls not at risk. Since this association was not due to adolescents’ experienced lifetime stress, the converted risk of parents to their daughters might, to a large extent, be the result of transmitted vulnerability genes. The amount and functioning of receptors involved in the activation and termination of the HPA axis are influenced by the genes that code for them (Holsboer et al., 2009).
Polymorphic glucocorticoid receptor genes have been associated with both altered HPA axis responses (Wüst et al., 2008; Kumsta et al., 2007; Chapter 6) and depression (van West et al., 2006, van Rossum et al., 2006).

Our findings should be interpreted in the light of the following strengths and limitations. To the best of our knowledge, we are the first to study the effect of PDS on offspring cortisol responses to an acute social stress test in a large (> 300) non-clinical sample. There are also limitations to this study. First, lifetime PDS were measured by questionnaires, and did not directly represent DSM-IV criteria. Second, the social stress test was the final task of the experimental session and we cannot be certain that the former tasks did not influence our findings. However, systematic bias in the associations is not likely, since the social stress test was by far the most stressful element of the laboratory session (Oldehinkel, unpublished data). A final limitation might be that the Groningen Social Stress Test is probably less stressful than the original Trier Social Stress Test. Despite this, evaluative threat and uncontrollability, the combination of which is assumed to induce a psychophysiological stress response (Dickerson and Kemeny, 2002), are both present in the GSST, and we did find observe cortisol responses, as well as meaningful differences therein.

This study is the first to show an association between PDS and cortisol responses to a standardised social stress test in adolescent girls in a non-clinical sample. It is important to determine how children and adolescents at familial risk for depression respond to stress, because it adds to the understanding of the transmission of depression across generations. It remains to be seen if the blunted response we observed is a mechanism through which familial risk for depression is transmitted to daughters. The prognostic value of blunted cortisol responses to social stress needs further exploration, preferably in longitudinal studies such as TRAILS. In the future, we will be able to explore if the girls who displayed blunted responses are more at risk to develop depressive symptoms or rather the opposite. Findings from genetic association studies in humans hopefully learn us more about which genes in which environments are important players in sensitivity to stress and the development of depressive symptoms. Future research may also shed more light on the intriguing gender difference in determinants of HPA axis functioning that was suggested by this study.