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Neuroendocrine stress response in female and male adolescents with Conduct Disorder and associated early adversity

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Abstract:	Objective: Conduct disorder (CD) involves aggressive and antisocial behavior and is associated with blunted cortisol responses to stress in male adolescents. However, far less is known about cortisol stress responsivity in females with CD or other neuroendocrine systems in both sexes. Although CD is linked to early adversity, the possibility that neuroendocrine system alterations mediate the relationship between early adversity and CD has not been systematically investigated.

	Method: Within the FemNAT-CD multi-site study, salivary cortisol, testosterone, and oxytocin levels as well as psychological stress in response to a standardized psychosocial stress test (the Trier Social Stress Test) were assessed in 130 pubertal adolescents with CD (63% female, 9-18 years) and 160 sex-, age-, and puberty-matched healthy controls. Common pre- and postnatal environmental risk factors were also assessed. Results: Females and males with CD both showed blunted cortisol, testosterone, and oxytocin responses to stress compared to healthy controls. In contrast, the CD group reported higher levels of psychological stress. Psychoneuroendocrine stress responses partly mediated the relationship between environmental risk factors and CD. Conclusion: Findings provide evidence for a widespread attenuated stress responsivity of not only the stress hormone, but also sex hormone and neuropeptide systems in CD. Our results are the first to demonstrate similarly blunted neuroendocrine stress response in both females and males with CD. Results of mediation analyses suggest a mechanism through which early adversity increases risk for CD . Considering neurobiological characteristics and associated environmental risks in personalizing an individual's treatment may help to improve future interventions for CD.

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To the Editor-in-chief
Douglas K. Novins, MD
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Manuscript submission to the *Journal of the American Academy of Child & Adolescent Psychiatry*

Dear Dr. Novins,

please find attached our manuscript *"Neuroendocrine stress response in female and male adolescents with Conduct Disorder and associated early adversity"* which we are submitting for publication in the *Journal of the American Academy of Child & Adolescent Psychiatry*.

Conduct Disorder (CD) is one of the most impairing, but also one of the most understudied mental disorders of childhood and adolescence, especially in females. CD is associated with blunted cortisol responses to stress in male adolescents. However, far less is known about stress responsivity in females with CD, or alterations in additional neuroendocrine systems in both sexes.

To our knowledge, our study is the largest in the literature to date (N=290) on neuroendocrine stress responsivity in CD compared to healthy controls, and the first to simultaneously investigate biomarkers of the HPA-axis (cortisol), HPG-axis (testosterone) and neuropeptide (oxytocin) systems next to psychological stress in response to a standardized psychosocial stress test (the Trier Social Stress Test) in both females and males with CD. Our study is the

first to demonstrate that females with CD show similarly attenuated HPA-axis responses to stress as males with CD, and that these alterations extend to the HPG-axis and neuropeptide systems in both sexes compared to healthy adolescents. Interestingly, an increased psychological stress response was observed in individuals with CD, suggesting poorer coordination of neuroendocrine and psychological stress responses in CD. Furthermore, this study explored whether alterations in neuroendocrine stress responses measures mediate the effects of early adversity on CD. Altered neuroendocrine responses to stress partly mediated the relationship between pre- and postnatal risk factors and CD suggesting a mechanism by which early adversity increases risk for CD.

Overall, our findings substantially extend our understanding of the neurobiological basis of CD and enhance knowledge about risk factors and mechanisms involved in CD. We believe our work will be of interest to a broad audience, such as the readership of the *Journal of the American Academy of Child & Adolescent Psychiatry*.

This manuscript and its data are original, have been contributed by the stated authors, have not been published previously and are not under consideration for publication elsewhere. All authors have approved the final submission.

Thank you very much for your consideration. We look forward to hearing from you.

Yours sincerely,

Dr. Anka Bernhard

Signed on behalf of all co-authors

Neuroendocrine stress response in female and male adolescents with Conduct Disorder and associated early adversity

Running head: Stress response in Conduct Disorder

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Disclosure: Professor Dr. Konrad has received speaker fees from Shire Pharmaceuticals and Medice and receives royalties for books. Professor Dr. Stadler receives royalties for a book on aggression. Professor Dr. Freitag receives royalties for books on Autism Spectrum Disorder, ADHD, and depressive disorder. Drs. Bernhard, Ackermann, Martinelli, Vllasaliu, Gonzalez-Madruga, Raschle, Jansen, Kohls, Popma and Fairchild, and Mss. Batchelor and Oldenhof have reported no biomedical financial interests or potential conflict of interest.

Neuroendocrine stress response in female and male adolescents with Conduct Disorder and associated early adversity

Running head: Stress response in Conduct Disorder

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Abstract

Objective: Conduct disorder (CD) involves aggressive and antisocial behavior and is associated with blunted cortisol responses to stress in male adolescents. However, far less is known about cortisol stress responsivity in females with CD or other neuroendocrine systems in both sexes. Although CD is linked to early adversity, the possibility that neuroendocrine system alterations mediate the relationship between early adversity and CD has not been systematically investigated.

Method: Within the FemNAT-CD multi-site study, salivary cortisol, testosterone, and oxytocin levels as well as psychological stress in response to a standardized psychosocial stress test (the Trier Social Stress Test) were assessed in 130 pubertal adolescents with CD (63% female, 9-18 years) and 160 sex-, age-, and puberty-matched healthy controls. Common pre- and postnatal environmental risk factors were also assessed.

Results: Females and males with CD both showed blunted cortisol, testosterone, and oxytocin responses to stress compared to healthy controls. In contrast, the CD group reported higher levels of psychological stress. Psychoneuroendocrine stress responses partly mediated the relationship between environmental risk factors and CD.

Conclusion: Findings provide evidence for a widespread attenuated stress responsivity of not only the stress hormone, but also sex hormone and neuropeptide systems in CD. Our results are the first to demonstrate similarly blunted neuroendocrine stress response in both females and males with CD. Results of mediation analyses suggest a mechanism through which early adversity increases risk for CD. Considering neurobiological characteristics and associated environmental risks in personalizing an individual's treatment may help to improve future interventions for CD.

Introduction

Conduct Disorder (CD) is characterized by aggressive, antisocial and rule-breaking behavior, and is one of the most impairing, but also one of the least studied mental disorders in childhood and adolescence, particularly in females [1]. While prevalence rates have increased in recent decades, studies on females with CD remain scarce [2]. A growing body of evidence indicates that CD is associated with neurobiological alterations, such as changes in brain, autonomic nervous system, or neuroendocrine functioning.

One of the major neurobiological characteristics associated with CD is altered hypothalamic-pituitary-adrenal (HPA)-axis activity [3]. The HPA-axis is essential for responding to and regulating the impact of stress. During stress, the hypothalamus releases corticotropin-releasing hormone, triggering adrenocorticotropin release from the pituitary. Adrenocorticotropin stimulates the adrenal gland to release cortisol into the bloodstream [4]. Salivary cortisol serves as a widely used peripheral marker of HPA-axis functioning [5]. Despite similar subjective emotional responses, lower salivary cortisol stress responses to public-speaking or anger-provocation tasks have been consistently reported in males with CD compared to healthy individuals (see [6] for review). Importantly, it is unknown whether females with CD show similarly attenuated HPA-axis responsivity to stress, although lower morning cortisol levels have been reported in girls with CD compared to healthy controls [7].

Notably, previous studies on neuroendocrine stress responsivity in CD focused on the HPA-axis, mainly by assessing cortisol. This is surprising given the complex interplay between the major neuroendocrine systems, which are assumed to work in a coordinated manner to enable successful stress regulation in both females and males [8–10]. For example, the hypothalamic-pituitary-gonadal (HPG)-axis interacts with the HPA-axis [4]. A corresponding stress responsivity of the HPA- and HPG-axes is assumed given the positive correlation of

cortisol and the sex hormone testosterone in response to stress in healthy children and adults [8]. While positive associations between basal testosterone, aggressive behavior and CD were reported [11], sex hormones have not been assessed under stressful conditions in CD. Additionally, neuropeptides (e.g., oxytocin) influence HPA-axis responsivity. Oxytocin inhibits HPA-axis activity, evoking stress-protective and anxiolytic effects [10]. Most stressors that stimulate HPA-axis activity also activate the oxytocin system [12]. Accordingly, peripheral salivary cortisol and oxytocin are co-activated under stress in healthy adolescents and adults, with oxytocin release preceding cortisol secretion [13,14]. While basal oxytocin system alterations have been reported [15], the oxytocin response to stress has not been investigated in adolescents with CD. Given the simultaneous activation of HPA-axis, HPG-axis, and neuropeptide systems in response to stress, it is important to apply a multi-system approach to understand the overarching neurobiology of stress regulation in CD.

In addition, given that CD is strongly associated with early adversity [16], findings of blunted neuroendocrine stress responsivity may be influenced by exposure to prenatal (e.g., maternal smoking or violence exposure) or postnatal risk factors (e.g., exposure to trauma or familial conflicts). Such early adversity has been associated with altered HPA-axis, HPG-axis and neuropeptide system functioning in adolescents and adults [17–19]. Attenuated neuroendocrine stress responsivity may represent an adaptation to early-life adversity which would otherwise overload the neuroendocrine systems [20]. A recent review highlighted the importance of considering the impact of adversities such as early-life trauma in neuroendocrine research on CD [21]. While no previous study investigated whether neuroendocrine stress responses mediate the relation between early adversity and CD, such work is strongly warranted to understand the neurobiological mechanisms that may underpin the association between early adversity and CD.

Overall, previous neuroendocrine research in CD focused on HPA-axis functioning in response to stress and neglected other related neuroendocrine systems as well as the possibility that the effects of early environmental risk for CD are mediated by neuroendocrine alterations. Critically, all previous research on neuroendocrine responsivity was limited to males with CD, thus almost nothing is known about stress responsivity in females with CD. To address these research gaps, we investigated HPA-axis (cortisol), HPG-axis (testosterone) and neuropeptide (oxytocin) responses to a standardized psychosocial stress test in males and females with CD compared to sex-matched healthy controls (HCs). Given previous evidence of cortisol hypo-reactivity in females and males with CD [6,7], and coordinated HPA-axis, HPG-axis and neuropeptide responsivity to stress [8,10], we hypothesized an overall attenuated neuroendocrine stress responsivity in females and males with CD compared to HCs. Additionally, we explored whether neuroendocrine stress responses mediated the relationship between common pre- and postnatal risk factors and CD.

Method

Participants

This study included 130 pubertal adolescents (9-18 years) with CD (82 females) and 160 healthy adolescents (104 females) from the European "Neurobiology and Treatment of Female Conduct Disorder" (FemNAT-CD) project [2]. Data were collected between 2014 and 2017 across five European sites (Germany, Switzerland, the Netherlands, and the UK; see Table S1). Most participants were born in the country of assessment (Mean [SD], %: adolescents 95.04 [2.96], parents 75.11 [8.94]). Local ethical committees at each site approved the study. Participants were recruited from clinics, youth offending services, schools, and the community (e.g., via newspaper adverts or social media). Written informed consent was obtained from all participants and/or their legal guardians after a detailed study description. Exclusion criteria included IQ<70, pre-pubertal status, pregnancy, last menstruation >6 months ago, history of neurological disorder, traumatic brain injury, schizophrenia, autism spectrum disorder, or current mania or bipolar disorder. Adolescents with CD fulfilled DSM-IV-TR criteria for current CD [22]. HCs had neither current DSM-IV-TR disorders nor a history of disruptive behavior- or attention-deficit/hyperactivity disorder (ADHD).

Procedures

Current and lifetime psychiatric disorders were assessed with the Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime version (K-SADS-PL, [23]), a semi-structured diagnostic interview conducted separately with adolescents and parents/caregivers by trained staff. Inter-rater reliability (Cohen's κ=.91) and agreement (95%) for CD were high (N=75). For comorbid psychiatric disorders (e.g., ADHD, oppositional defiant disorder, major depressive disorder [MDD)] Posttraumatic Stress Disorder [PTSD]) inter-rater reliabilities (Cohen's κ=.50-.95) and agreements (92-95%) were

moderate to high. IQ was estimated from the matrix reasoning and vocabulary subtests of the Wechsler Intelligence Scale-IV (11-16 years [24]; >16 years [25]). The UK site used the Wechsler Abbreviated Scale of Intelligence [26]. Parental educational status was defined as the mean of the highest maternal and paternal self-reported school or occupational degree following International Standard Classification of Education (ISCED) criteria [27]. ISCED criteria for parental educational status are based on six categorical levels: 0=pre-primary level of education, 1=primary level of education, 2=lower secondary level of education, 3=upper secondary level of education, 4=post-secondary level of education, 5=first stage of tertiary education, 6=second stage of tertiary education. Pubertal status was assessed using the Pubertal Development Scale [28], a self-report measure of pubertal growth (e.g. changes in body hair, voice or breast development) with four response options (not yet, barely or definitely started, seems complete) resulting in a five-level categorical scale (0=pre-pubertal, all items not yet started; 1=early-pubertal, at least one item barely started; 2=mid-pubertal, at least one item definitely started; 3=late-pubertal, all items definitely started, 4=post-pubertal, all items seems complete). The internal consistency was α=0.77 [28]. Parents/caregivers reported on four widely replicated pre- and postnatal risk factors: prenatal smoking and violence exposure (yes/no), adverse family situation (summary score of familial disharmony and isolation due to correlation r>0.3, three categories: 0=zero yes responses, 1=yes to either disharmony or isolation, 2=yes to both items), and the number of DSM-IV-TR traumatic events experienced (based on 11 potentially traumatic experiences from the K-SADS-PL PTSD section, e.g., life-threatening accidents, disasters, or physical/sexual abuse, summary score self- and parent-rating).

Psychoneuroendocrine assessment

Stress responsivity (see Figure 1) was assessed with the Trier Social Stress Test (TSST) [29], a widely-used, standardized method for inducing psychosocial stress in laboratory settings [30].

After a 60- minute relaxation period in a comfortable room (Room A) to minimize possible effects of previous food/drink intake, exercise, or stressful events, the baseline assessments were taken. Next, participants entered a sparsely-equipped experimental room (Room B) and were introduced to the task. After a short preparation period, they had to give a public speech (about their favorite movie including the storyline and their opinions of the characters and plot), followed by an age-adapted mental arithmetic task (serial subtraction of a fixed number from a given starting number, e.g., 13 from 1023, starting again from the beginning if they gave the wrong answer). Both tasks were performed in front of two unfamiliar panel members and a video camera. Post-stress assessments were completed in Room A. Participants were given positive feedback about their performance, informed that no recordings were made, and fully debriefed. Alcohol/substance use was not permitted on the assessment day nor drinking, eating, or smoking during the whole experiment. To confirm stress induction, participants rated their feelings of stress ("Do you feel stressed?") eight times from baseline to +55 minutes after stress termination using a Visual Analog Scale (range 0-10) [31]. Saliva samples were collected using Salivettes (Sarstedt, Germany) at baseline and +1, +10, +25, +40 and +55 minutes after stress termination. Samples were stored at -20°C until analysis.

[Figure 1]

Levels of salivary cortisol (nmol/l) at baseline, +10, +25, +40 and +55 minutes, and testosterone (pg/ml) at baseline, +10 and +55 minutes were analyzed by Daacro (Trier, Germany) employing an enzyme immunoassay kit (Salimetrics, LLC, USA) formatted to minimize cross-reactivity for related steroids. The sensitivity limit was 0.19-82.77 nmol/l for cortisol and 1-600 pg/ml for testosterone. The corresponding inter- and intra-assay coefficients of variation were in the commonly accepted range (<15% and 10%, respectively [32]). After the cortisol and testosterone analyses, the baseline, +1 and +10 minutes salivettes were transferred to RIAgnosis (Sinzing, Germany) for quantification of salivary oxytocin

(pg/ml) by radioimmunoassay as described previously [13]. For each sample, 300 µl of saliva was evaporated (Concentrator, Eppendorf, Germany) and 50µl of assay buffer was added followed by 50µl rabbit antibody against oxytocin. The detection limit was in the 0.5 pg/sample range. Intra- and inter-assay coefficients of variation were <10%. Oxytocin was measured in a sub-sample of 170 participants (for site distribution see Table S1), including 80 adolescents with CD (45 females) and 90 HCs (49 females), as collection of samples at the +1 minutes timepoint (optimal for oxytocin) commenced later than the other timepoints. Different timepoints for cortisol, testosterone and oxytocin analyses were used based on reported distinct reactivity patterns [14,30].

Statistical Analyses

Statistical analyses were performed using SPSS v24 (IBM Corp., Armonk, NY). Significance levels of all tests were set at $p \leq 0.05$ (two-tailed). The groups' demographic and clinical characteristics were compared using univariate analyses of variance or Chi-Squared-tests. Neuroendocrine analyses controlled for age, current smoking, body mass index (BMI), TSST start time, medication (incl. contraceptive) use, and site; psychological stress analyses for age and site.

Neuroendocrine data was complete for all assessed timepoints of all participants. First, psychoneuroendocrine stress responses were analyzed using repeated measures analyses of co-variance on the dependent variables cortisol, testosterone, oxytocin, and psychological stress with group (CD vs. HCs) and sex (female vs. male) as between-subjects factors, and time as a within-subject factor. Significant main or interaction effects were followed by post-hoc Bonferroni-corrected pairwise comparisons. Neuroendocrine measures were log-transformed to normalize their distribution. For easier interpretation, Figure 1 shows the untransformed data. Dimensional covariates (age, BMI, TSST start time) were mean-centered [33]. Greenhouse-Geisser corrections were applied as required, but for clarity uncorrected

degrees of freedom are reported. Effect sizes are described using partial eta squared η^2_p with 0.01 representing a small, 0.06 a medium and 0.14 a large effect [34]. Our sample size enabled us to detect even small, including group-by-sex interaction effects, with a power of 80% and a two-sided significance level of 5% (G*Power 3.1.9.2). To exclude confounding, the influence of IQ, pubertal status, and psychiatric comorbidities (ADHD, MDD, PTSD, substance use disorder (SUD), anxiety disorders (ANX)) was controlled for in additional separate analyses. Correlations between CD age-of-onset and CD severity with area under the curve measures (see below) for cortisol, testosterone, oxytocin, and psychological stress were calculated using Spearman-correlations.

Second, to explore mediating effects of psychoneuroendocrine responses (M) on associations between pre- or post-natal risk factors (X) and CD status (Y), mediation analyses were performed using the PROCESS macro (http://www.processmacro.org/). Therefore, aggregated psychoneuroendocrine stress response measures were computed [14]. As a routinely used measure for overall stress response, the area under the curve with respect to increase (AUC_I), referring to the baseline level, was calculated. To explore stress reactivity and stress regulation separately, stress-increase (difference baseline-peak) and post-stress recovery (difference peak-lowest level after stress termination) were calculated. For each risk factor and possible mediator (aggregated psychoneuroendocrine stress measures), a mediation analysis was run yielding direct, indirect, and total effects. These were: a) a linear regression model of the mediator as a function of the risk factor (direct effect X→M), b) a logistic regression model of the case-control-status as a function of the mediator (direct effect M→Y) as well as of the risk factor (direct effect X→Y), and c) a logistic regression model of the case-control-status as a function of the risk factor (indirect effect X→M→Y). The standardized estimates of the effects are reported together with the proportion of the total effect mediated by each measure. Applying bootstrapping with 1000 resamples, percentile

bootstrap confidence intervals for the indirect effects were computed. The null hypothesis of no mediation effect was rejected if the 95% confidence interval did not include 0 [35]. As the mediation analyses tested for associations between risk factors and psychoneuroendocrine stress measures in an exploratory fashion, results are presented descriptively without correction for multiple comparisons.

Results

Sample characteristics

Groups did not significantly differ in sex, age, pubertal status, TSST start time, nor in baseline cortisol, testosterone, and oxytocin levels or psychological stress ratings. Participants with CD had higher BMIs and rates of current smoking and medication use, but lower IQ and parental educational status than HCs. Participants with CD had higher rates of risk factors and psychiatric comorbidities than HCs (see Table 1). Most female participants had started menstruating (CD 92%, HCs 79%) with comparable days since the last menstruation (Mean [SD]: CD 21.16 [18.22], HCs 20.09 [14.15], p=.71). Results for the oxytocin subsample were similar (see Table S2).

[Table 1]

Repeated-measures analyses of psychoneuroendocrine stress responses

The TSST induced significant neuroendocrine stress responses in HC, but not CD participants, with the two groups differing for all neuroendocrine measures (see Figure 2, Table 2). As evidenced by significant group-by-time interaction effects followed up using post-hoc Bonferroni-corrected pairwise comparisons, neither cortisol, nor testosterone or oxytocin increased in response to stress in participants with CD, whereas strong increases in all three neuroendocrine measures were observed in HCs. Regarding sex, similar attenuations of cortisol, testosterone, and oxytocin responses were found in females and males with CD (no group-by-sex-by-time interaction effects, except for testosterone indicating a sex effect only in HCs) while testosterone was generally higher in males and oxytocin higher in females.

In contrast, the TSST induced a significant psychological stress response in CD and HC participants (main effect of time). Interestingly, adolescents with CD reported higher overall psychological responses than HCs (significant group effect). Bonferroni-corrected

post-hoc comparisons indicated slower psychological post-stress recovery in CD participants than HCs, with higher levels for the post-stress assessments (see Figure 2). No group-by-time interaction effect was found, indicating similar psychological responses to the TSST in both groups. In addition, no interactions with sex were observed, suggesting increased psychological stress responses in both females and males with CD relative to HCs.

[Table 2, Figure 2]

Additional analyses for potential confounders on psychoneuroendocrine stress responses

Repeating the analyses with IQ included as a covariate did not affect significance of the results for cortisol, oxytocin, and psychological stress, except for the group-by-time interaction for testosterone which was only marginally significant in the respective model [$F(2,558)=2.95$, $p=.06$, $\eta^2_p=0.01$]. Controlling for pubertal status did not impact the findings besides reducing the strength of the main effect of sex on oxytocin [$F(1,159)=2.66$, $p=.11$, $\eta^2_p=0.02$]. Psychiatric comorbidities did not affect cortisol and oxytocin results, while comorbid ADHD, SUD and MDD rendered the group-by-time interactions for testosterone marginally significant ($p=.06$-$.10$), and ADHD, ANX and MDD the group effect for psychological stress ($p=.05$-$.13$). CD age-of-onset did not correlate with any stress responsivity measure ($r<-.13$, $p>.18$), while CD severity correlated negatively with the AUC_I for cortisol ($r=-.37$, $p<.001$), testosterone ($r=-.24$, $p<.001$), and positively with psychological stress ($r=.16$, $p=.01$).

Exploratory mediation analyses of environmental risk for CD by psychoneuroendocrine stress responses

In additional exploratory analyses (see Figure 3, Table S3), AUC_I of cortisol was found to mediate the relation between prenatal violence exposure and adverse family situation with CD status, explaining 7-10% of the total effect. Similar mediation effects were observed for cortisol increase (13-15% of the total effect). Cortisol recovery mediated the relationships between prenatal smoking, adverse family situation and trauma exposure with CD status (13-

16% of the total effect). Oxytocin increase mediated the relation between prenatal smoking and CD, and oxytocin recovery between prenatal smoking and violence with CD (10-13% of the total effect). The AUC_I for psychological stress mediated the relation between adverse family situation and CD (5% of the total effect). No other significant mediating effects were found.

[Figure 3]

Discussion

To our knowledge, this is the first and the largest study to date which comprehensively examines whether females with CD show similarly attenuated HPA-axis stress responses compared to males with CD, and also the first to simultaneously investigate other neuroendocrine systems. Our results replicate and considerably extend previous work of smaller studies in males with CD focusing on cortisol stress response by providing evidence for a diminished neuroendocrine stress responsivity of not only the HPA-axis, but also the HPG-axis and neuropeptide systems in CD. Critically, this neuroendocrine hypo-reactivity to stress was observed in both females and males with CD compared to healthy adolescents. Furthermore, changes in psychoneuroendocrine stress responsivity mediated the relationship between common pre- and postnatal risk factors and CD, suggesting that exposure to early adversity may increase risk for CD partly through its effects on the body's neuroendocrine systems. All analyses controlled for relevant confounders such as smoking, BMI, TSST start time, or medication use (incl. contraceptives). IQ and pubertal status did not substantially affect results. The female CD and HC groups did not differ in menstrual cycle status with most girls being in the luteal phase during which the neuroendocrine stress response is more comparable to that observed in males [36].

Individuals with CD differed substantially in their psychoneuroendocrine stress responsivity compared to healthy adolescents. While HCs showed clear cortisol, testosterone, and oxytocin responses to the TSST (comparable to previous reports in healthy adolescents [8,14,30]), a widespread attenuation of neuroendocrine stress responsivity was found in CD. In line with earlier reports [6], we observed blunted cortisol stress responsivity in males with CD. Importantly, our results demonstrate that females with CD show similarly attenuated cortisol stress responses as males with CD. As earlier work did not investigate females and males with CD in response to a standardized stress task, our findings are the first to indicate blunted

HPA-axis activity as a distinct characteristic of CD independent of sex. Furthermore, as earlier studies on neuroendocrine stress responsivity in CD were restricted to cortisol, our results substantially extend previous work by showing comparable attenuations for testosterone and oxytocin in both females and males with CD. In line with previous findings [37], CD age-of-onset did not influence results. In contrast, as CD severity increased, psychoneuroendocrine stress responses were more strongly altered (blunted) similar to earlier reports [38,39] supporting dimensional approaches to understanding CD. Attenuated cortisol and oxytocin stress responses were not explained by psychiatric comorbidities such as ADHD, PTSD, anxiety, depressive or substance use disorders.

Our findings of concurrently attenuated steroid hormone and neuropeptide stress responses suggest an overall impaired neuroendocrine stress-regulation in females and males with CD, which may also impair the individual's ability to cope with stressful situations. Our findings of higher psychological stress responsivity in CD compared to HCs support this notion. Coordinated stress responsivity of the HPA-axis, HPG-axis and neuropeptide systems may facilitate successful management of stressful situations with cortisol activating energy resources, testosterone improving performance, and oxytocin reducing feelings of stress and anxiety [8,14]. As the groups did not differ in baseline psychological stress, our findings suggest individuals with CD to be more psychologically affected by stress than HCs. Similar or higher rates of negative feelings in children and adolescents with CD in response to stress have been reported previously [6]. Notably, results of post-hoc-comparisons in this study indicate slower recovery from psychological stress in CD compared to healthy individuals. The psychological stress response appears to be more prolonged in individuals with CD, persisting beyond the TSST. Attenuated neuroendocrine stress responsivity may prevent successful adaptation to stress as this may normally help the individual to regulate their levels of psychological stress. This may relate to emotion regulation difficulties, which have been reported in CD [40].

Interestingly, a similar discrepant pattern of psychoneuroendocrine stress responsivity to the TSST (decreased cortisol, but increased psychological responses) was found in adult females with borderline personality disorder, which is characterized by severe emotion dysregulation [41].

Given the widespread psychoneuroendocrine alterations observed in CD, understanding their origins is important. Exposure to adversity has been reported to attenuate neuroendocrine stress responsivity [17–19] and has also been strongly associated with CD [16], as replicated here. Thus, we applied mediation analyses to explore the influence of psychoneuroendocrine stress responses on the relation between pre- and post-natal risk factors and CD. Mediation effects were found for cortisol and oxytocin (both particularly relevant for stress regulation) as well as psychological stress responses. Exposure to prenatal smoking/violence, adverse family situation, and trauma not only predicted CD, but also attenuated cortisol and oxytocin accompanied by higher psychological stress responses. These alterations, in turn, predicted CD. Our results indicate neurobiological alterations in CD, such as attenuated psychoneuroendocrine stress responses, are associated with early adversity. However, given the exploratory design, results need to be replicated. Yet, our findings are in line with reports of similar neurobiological mediation effects of structural brain alterations on the relation between early adversity and antisocial behavior in adolescents [42]. Attenuated neuroendocrine stress responsivity may constitute an underlying mechanism by which environmental risks contribute to CD risk driven by an early adaptation to adversity [20]. However, the underlying neural mechanisms, e.g., alterations in brain structure or functioning, need to be identified in future studies.

Considering impairments in psychoneuroendocrine stress regulation and associated environmental risk factors in the process of treating CD may help to improve individuals' stress coping strategies, which may reduce the incidence of CD-related behaviors. In line with

this, cortisol responsivity has been shown to predict responses to behavioral interventions in children and adolescents with disruptive behavioral disorders, with blunted stress responsivity linked to poorer treatment outcomes [43]. To improve outcomes of interventions for CD, it may be possible to personalize treatments based on an individual's neurobiological characteristics, e.g., by implementing additional pharmacological treatments in those with a high neurobiological risk. However, effects of personalized interventions and pharmacological treatments seeking to normalize neuroendocrine hypo-reactivity are yet to be studied in adolescents with CD.

This study has several strengths. We included a large and representative European sample of females and males with and without CD, the groups being matched for sex, age, and pubertal status, allowing sex-specific analyses. Participants with CD and healthy controls were reliably diagnosed using standardized semi-structured diagnostic interviews according to DSM-IV-TR criteria. Our study is the first to date which investigated psychoneuroendocrine stress responses in females compared to males with CD applying a standardized and widely used laboratory psychosocial stress test procedure (the TSST [29,30]). Moreover, we reliably assessed established biomarkers of three major neuroendocrine systems, namely the HPA-axis (cortisol), HPG-axis (testosterone) and neuropeptide (oxytocin) system, while previous work in males with CD has focused on cortisol reactivity alone. Importantly, we also controlled for major confounding variables on neuroendocrine functioning (e.g. smoking, BMI, TSST start time, or medication use). Finally, this is the first study exploring whether neuroendocrine markers mediate the effects of environmental risk factors for CD.

However, our study also has several limitations. First, data were collected across five European sites, and the sites contributed different sample sizes according to site-specific recruitment possibilities. To ensure comparability, standardized operating procedures were used at each site during all assessments with adherence controlled by external monitors. Site

was also included as a covariate in the analyses. Second, the age range was relatively large. Nevertheless, groups were matched for age, and age was included as a covariate. Third, data on oxytocin stress responsivity was obtained in a smaller sample compared to our cortisol and testosterone measures, but still in a relatively large sample (N=170) compared to other neuroendocrine studies. Further, while steroid hormones can pass the blood-brain-barrier, non-invasive peripheral salivary assessment may not fully capture central release of oxytocin. However, salivary oxytocin has been repeatedly reported as a valid, non-stressful (which is especially relevant for research on stress responsivity) assessment in adolescents and adults [13,14] correlating with its central concentrations [44]. Fourth, the only sex hormone studied here was testosterone, a predominantly male hormone, as work investigating female sex hormone responses to stress in children and adolescents is rare. Although concentrations are lower than in males, testosterone is still an essential part of the female neuroendocrine system. Fifth, storage of saliva samples at -20°C was reported as less optimal than -80°C [45]. However, as storage was similar across groups, possible degradation by storage temperature may only have influenced absolute, rather than relative concentrations. Sixth, pubertal status was assessed via self-report. Future studies may consider additional measures of pubertal development, such as physical examination by pediatricians. Seventh, CD is a heterogeneous disorder characterized by different subtypes (e.g., callous-unemotional traits, childhood- versus adolescent-onset, reactive, proactive, and/or relational aggression) [46–48], which may influence neuroendocrine characteristics [49,50]. Further studies are needed to investigate the impact of different CD subtypes, although CD severity rather than age-of-onset appeared more influential in determining stress responsivity here. Finally, pre- and postnatal risk factors were assessed retrospectively, predominantly relying on parent-report data, and mediation analyses were performed only in an exploratory fashion. Further, at best prospective longitudinal studies investigating overlapping questions are thus needed.

In conclusion, this work substantially extends current knowledge about stress responsivity in CD beyond males. It is the first and largest study providing compelling evidence for attenuated stress responsivity in not only males, but also females with CD. Our study not only demonstrates reduced cortisol stress responsivity, but also testosterone and oxytocin hypo-reactivity, in both females and males with CD compared to healthy adolescents. Our results indicate a substantially altered neuroendocrine stress response in CD irrespective of sex. These findings were unrelated to potential confounding factors such as smoking, BMI, TSST start time, medication use (incl. contraceptives), IQ, pubertal status, CD age-of-onset, or site. The discrepancy between lower neuroendocrine, but elevated psychological stress responsivity suggests poorer coordination of neurobiological and emotional processes in CD. Our findings further suggest that neuroendocrine alterations may act as a possible underlying mechanism through which early adversity contributes to risk for CD. Considering neurobiological characteristics and associated environmental risks in personalizing an individual's treatment may help to improve future interventions for CD.

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Table/Figure legends

Table 1. Sample characteristics, risk factors, comorbidities, and baseline psychoneuroendocrine levels in conduct disorder (CD) participants compared to healthy controls (HCs).

Note. [a]For definition of pubertal categories, see *Methods.* [b]Non-stimulants include atomoxetine medication. 'Other' includes asthma medication, painkiller, or vitamin preparation. Participants may have been prescribed more than one medication category. [c]Rates of lifetime anxiety disorder cover lifetime panic disorder, separation anxiety disorder, avoidant disorder, simple phobia, social phobia, agoraphobia, overanxious disorder, and generalized anxiety disorder. [d]According to the trauma definition within the PTSD section of the K-SADS-PL (for further details see *Methods*).

Table 2. Results of repeated-measures ANCOVA of psychoneuroendocrine stress responses per group and sex.

Note. Repeated measure analyses of co-variance (rmANCOVA) with group (CD vs. HCs) and sex (female vs. male) as between-subjects factors, and time as a within-subject factor, respectively, controlled for major confounders (see Statistical Analyses). Where necessary, Greenhouse Geisser corrections were applied, but for clarity uncorrected degrees of freedom are reported here. η^2_p=partial eta squared. [a]Bonferroni-corrected post-hoc pairwise comparisons indicated a slower rate of recovery from stress in male compared to female HCs (Mean change +10 to +55 minute [SD]: female HCs 0.16 [0.03], p<.001, male HCs -0.002 [0.04], p=1.00, no significant differences between females and males with CD, p>.22). CD=Conduct Disorder, HCs=Healthy Controls.

Figure 1. Overview of stress test procedure.

Note. After a relaxation period in a comfortable room (Room A), the baseline assessment was taken, before participants entered a sparsely equipped experimental room (Room B). The Trier Social Stress Test (TSST) involved four components: task introduction, preparation time, speech task, and an age-adapted mental arithmetic task. For more information see *Methods*. CORT=Cortisol, OXT=Oxytocin, TEST=Testosterone, VAS=Visual Analogue Scale.

Figure 2. Psychoneuroendocrine stress responses during the Trier Social Stress Test in conduct disorder (CD) participants compared to healthy controls (HCs).

Note. fCD=female CD, fHCs=female HCs, mCD=male CD, mHCs=male HCs. Significant difference of respective value between CD and HCs: *p<.05, **p<.01, ***p<.001

Figure 3. Psychoneuroendocrine stress measures as mediators of the relation between early environmental risk factors and conduct disorder (CD).

Note. Numbers in brackets refer to 95% confidence intervals. *p<.05, **p<.01, ***p<.001. AUC_I=area under the curve with respect to increase.

Table 1. Sample characteristics, comorbidities, risk factors, and baseline psychoneuroendocrine levels in conduct disorder (CD) participants compared to healthy controls (HCs).

	Females (N=186)		Males (N=104)				
	CD (N=82)	HCs (N=104)	CD (N=48)	HCs (N=56)	Group	Sex	Group x sex
	Mean (SD) or N (%)				*p*	*p*	*p*
Age (years)	14.83 (1.5)	14.58 (2.1)	14.54 (2.1)	14.88 (2.1)	.87	.98	.22
Estimated Full-Scale IQ	97.32 (12.8)	104.33 (12.1)	101.21 (12.6)	105.75 (11.7)	<.001	.08	.42
Parental educational status	3.00 (1.0)	3.88 (1.1)	3.30 (0.7)	3.65 (0.9)	<.001	.78	.04
Body Mass Index	23.63 (6.5)	20.67 (3.9)	22.07 (4.0)	20.92 (3.6)	<.01	.27	.13
Start time Trier Social Stress Test (hh:mm)	15:08 (00:58)	15:16 (00:53)	15:07 (00:51)	14:56 (00:51)	.87	.12	.17
Pubertal Status[a]					.13	<.001	
Early-pubertal	1 (1.2)	2 (1.9)	10 (20.8)	12 (21.4)			
Mid-pubertal	5 (6.1)	19 (18.3)	13 (27.1)	17 (30.4)			
Late-pubertal	57 (69.5)	54 (51.9)	23 (47.9)	24 (42.9)			
Post-pubertal	19 (23.2)	29 (27.9)	2 (4.2)	3 (5.4)			
Current smoking	51 (62.2)	7 (6.7)	25 (52.1)	9 (16.1)	<.001	.79	
Any medication[b]	48 (58.5)	12 (11.5)	10 (20.8)	2 (3.6)	<.001	<.001	
Neuroleptics	5 (6.1)	0 (0.0)	3 (6.3)	0 (0.0)	<.01	.92	
Stimulants	12 (14.6)	0 (0.0)	5 (10.4)	0 (0.0)	<.001	.57	
Non-stimulants	0 (0.0)	0 (0.0)	2 (4.2)	0 (0.0)	.12	.06	
SSRIs/antidepressants	5 (6.1)	0 (0.0)	2 (4.2)	0 (0.0)	<.01	.68	
Contraceptives	32 (47.1)	10 (13.5)	0 (0.0)	0 (0.0)	<.001		
Other	9 (11.0)	7 (6.0)	2 (4.2)	2 (3.6)	<.05	.35	
Comorbidities							
Lifetime ADHD	31 (37.8)	0 (0.0)	28 (58.3)	0 (0.0)	<.001	.04	
Lifetime substance use disorder	18 (22.0)	0 (0.0)	14 (29.2)	1 (1.8)	<.001	.22	
Lifetime anxiety disorder[c]	18 (22.0)	2 (1.9)	17 (35.4)	2 (3.6)	<.001	.07	
Lifetime depressive disorder	35 (42.7)	4 (3.8)	9 (18.8)	0 (0.0)	<.001	<.01	
Lifetime PTSD	11 (13.4)	1 (1.0)	5 (10.4)	1 (1.8)	<.001	.82	
Pre- and postnatal risk factors							
Prenatal smoking	27 (40.9)	15 (16.1)	19 (48.7)	8 (15.7)	<.001	.54	
Prenatal violence	17 (25.4)	5 (5.4)	7 (18.4)	2 (2.4)	<.001	.42	
Adverse family situation					<.001	.99	
Either disharmony or isolation	25 (39.1)	16 (17.0)	11 (29.7)	12 (24.5)			
Both disharmony and isolation	20 (31.3)	3 (3.2)	11 (29.7)	1 (2.0)			
Number of traumatic events experienced[d]	2.78 (2.0)	1.08 (1.2)	2.13 (1.9)	1.46 (1.2)	<.001	.49	<.01
Baseline psychoneuroendocrine levels							
Cortisol (nmol/l)	2.91 (1.3)	2.64 (1.8)	3.22 (2.0)	3.35 (2.1)	.74	.02	.36
Testosterone (pg/ml)	39.64 (17.1)	34.30 (14.2)	60.40 (31.3)	58.12 (32.0)	.17	<.001	.59
Oxytocin (pg/ml)	1.37 (0.5)	1.39 (0.7)	1.07 (0.3)	1.27 (0.6)	.20	02	.34
Psychological stress	1.51 (2.5)	1.21 (2.0)	1.43 (1.7)	0.94 (1.7)	.11	.48	.69

Table 2. Results of repeated-measures ANCOVA of psychoneuroendocrine stress responses per group and sex.

	Cortisol				Testosterone				Oxytocin				Psychological stress			
mANCOVA	*df*	*F*	*p*	η^2_p	*df*	*F*	*p*	η^2_p	*df*	*F*	*p*	η^2_p	*df*	*F*	*p*	η^2_p
Time	4, 1120	6.45	<.01	0.02	2, 560	6.65	<.01	0.02	2, 320	6.33	<.01	0.04	7, 1988	31.51	<.001	0.10
Group	1, 280	11.55	<.01	0.04	1, 280	0.13	.72	0.00	1, 160	1.03	.31	0.00	1, 284	8.21	<.01	0.03
Sex	1, 280	0.03	.87	0.00	1, 280	40.39	<.001	0.13	1, 160	6.68	.01	0.04	1, 284	2.61	.11	0.00
Group x time	4, 1120	11.08	<.001	0.04	2, 560	3.90	.02	0.01	2, 320	4.28	.02	0.03	7, 1988	0.42	.76	0.00
Sex x time	4, 1120	1.73	.17	0.00	2, 560	1.67	.19	0.00	2, 320	0.65	.52	0.00	7, 1988	1.93	.12	0.00
Group x sex x time	4, 1120	1.28	.28	0.00	2, 560	4.30	.02[a]	0.02	2, 320	0.08	.92	0.00	7, 1988	0.76	.53	0.00

MANUSCRIPT SUBMISSION FORM—*JOURNAL OF THE AMERICAN ACADEMY OF CHILD AND ADOLESCENT PSYCHIATRY and JAACAP Connect*

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Section 2: Manuscript Information.

Manuscript Title: Neuroendocrine stress response in female and male adolescents with Conduct Disorder and associated early adversity

Complete Author List (in order): Anka Bernhard, Katharina Ackermann, Anne Martinelli, Leonora Vllasaliu, Karen Gonzalez-Madruga, Molly Batchelor, Nora Raschle, Helena Oldenhof, Lucres Jansen, Gregor Kohls, Kerstin Konrad, Arne Popma, Christina Stadler, Graeme Fairchild & Christine M. Freitag

Corresponding Author Name: Anka Bernhard

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Figure 1

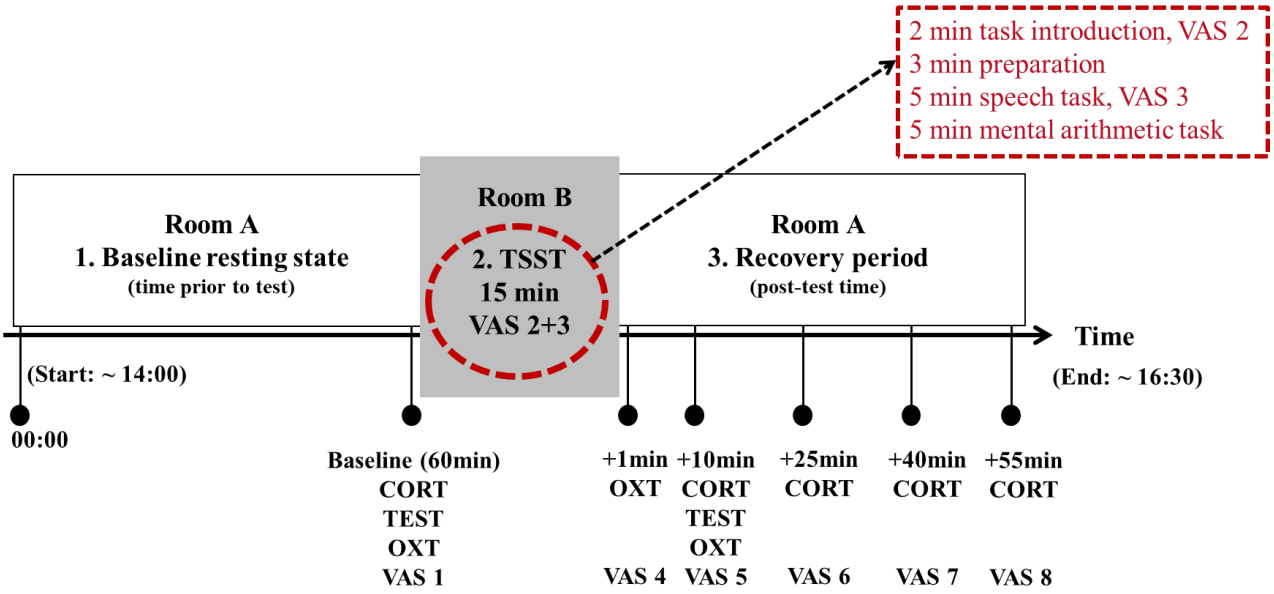

2 min task introduction, VAS 2
3 min preparation
5 min speech task, VAS 3
5 min mental arithmetic task
Room A
1. Baseline resting state
(time prior to test)
Room B
2. TSST
15 min
VAS 2+3
Room A
3. Recovery period
(post-test time)
Time
(Start: ~ 14:00)
(End: ~ 16:30)
00:00
Baseline (60min)
CORT
TEST
OXT
VAS 1
+1min
OXT
VAS 4
+10min
CORT
TEST
OXT
VAS 5
+25min
CORT
VAS 6
+40min
CORT
VAS 7
+55min
CORT
VAS 8

Figure 2

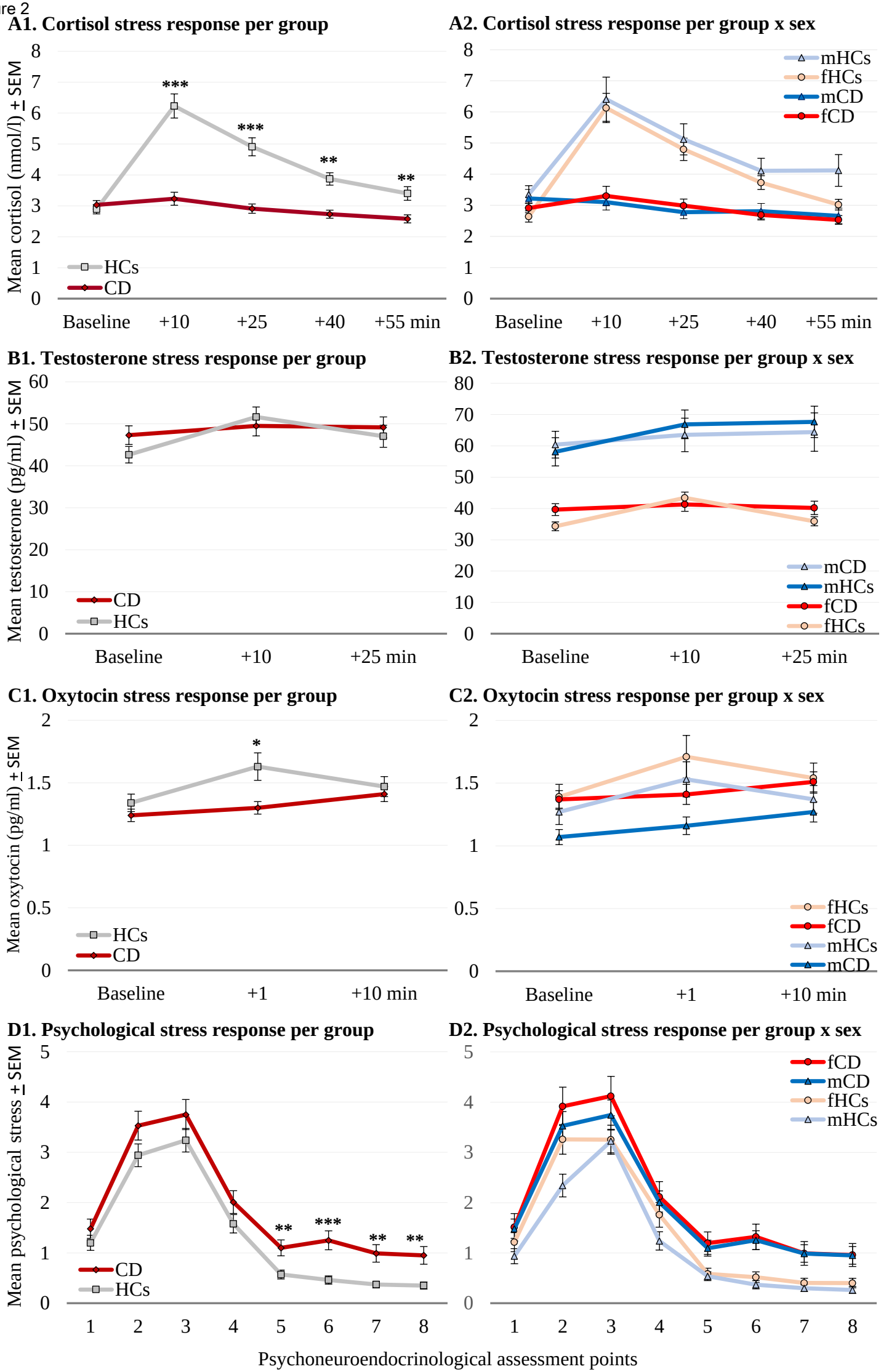
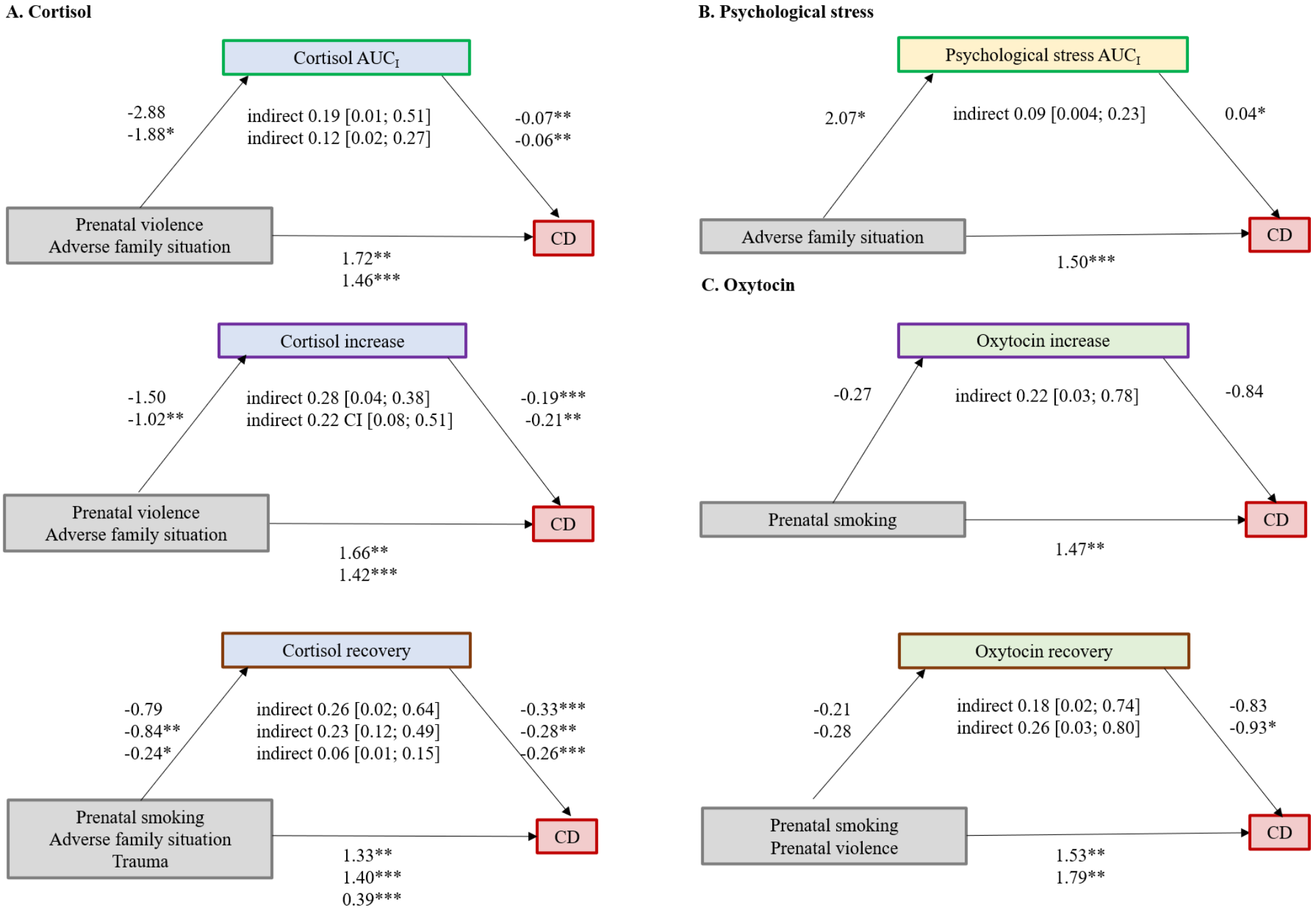

A1. Cortisol stress response per group
Mean cortisol (nmol/l) ± SEM
HCs
CD
Baseline
+10
+25
+40
+55 min
A2. Cortisol stress response per group x sex
mHCs
fHCs
mCD
fCD
B1. Testosterone stress response per group
Mean testosterone (pg/ml) ± SEM
CD
HCs
Baseline
+10
+25 min
B2. Testosterone stress response per group x sex
mCD
mHCs
fCD
fHCs
C1. Oxytocin stress response per group
Mean oxytocin (pg/ml) ± SEM
HCs
CD
Baseline
+1
+10 min
C2. Oxytocin stress response per group x sex
fHCs
fCD
mHCs
mCD
D1. Psychological stress response per group
Mean psychological stress ± SEM
CD
HCs
D2. Psychological stress response per group x sex
fCD
mCD
fHCs
mHCs
Psychoneuroendocrinological assessment points

Figure 3


A. Cortisol
Cortisol AUC$_I$
-2.88
-1.88*
indirect 0.19 [0.01; 0.51]
indirect 0.12 [0.02; 0.27]
-0.07**
-0.06**
Prenatal violence
Adverse family situation
CD
1.72**
1.46***
Cortisol increase
-1.50
-1.02**
indirect 0.28 [0.04; 0.38]
indirect 0.22 CI [0.08; 0.51]
-0.19***
-0.21**
Prenatal violence
Adverse family situation
CD
1.66**
1.42***
Cortisol recovery
-0.79
-0.84**
-0.24*
indirect 0.26 [0.02; 0.64]
indirect 0.23 [0.12; 0.49]
indirect 0.06 [0.01; 0.15]
-0.33***
-0.28**
-0.26***
Prenatal smoking
Adverse family situation
Trauma
CD
1.33**
1.40***
0.39***
B. Psychological stress
Psychological stress AUC$_I$
2.07*
indirect 0.09 [0.004; 0.23]
0.04*
Adverse family situation
CD
1.50***
C. Oxytocin
Oxytocin increase
-0.27
indirect 0.22 [0.03; 0.78]
-0.84
Prenatal smoking
CD
1.47**
Oxytocin recovery
-0.21
-0.28
indirect 0.18 [0.02; 0.74]
indirect 0.26 [0.03; 0.80]
-0.83
-0.93*
Prenatal smoking
Prenatal violence
CD
1.53**
1.79**

Supplemental information for

"Neuroendocrine stress responses in female and male adolescents with Conduct Disorder and associated early adversity"

Table S1. Number of participants per group and sex across sites.

	Females		Males				
	CD	HCs	CD	HCs	∑	group	sex
	N	N	N	N	N	*p*	*p*
Total sample						.01	<.001
Frankfurt am Main, Germany	29	49	34	32	144		
Aachen, Germany	13	2	3	3	21		
Amsterdam, The Netherlands	15	14	0	0	29		
Southampton, United Kingdom	16	16	8	16	56		
Basel, Switzerland	9	23	3	5	40		
∑	82	104	48	56	290		
Oxytocin subsample						.08	<.001
Frankfurt am Main, Germany	26	30	29	27	112		
Aachen, Germany	8	0	2	3	13		
Amsterdam, The Netherlands	7	14	0	0	21		
Southampton, United Kingdom	4	5	2	8	19		
Basel, Switzerland	0	0	2	3	5		
∑	45	49	35	41	170		

Note. CD=Conduct Disorder, HCs=healthy controls.

Table S2. Sample characteristics, comorbidities, and risk factors in Conduct Disorder (CD) participants compared to healthy controls (HCs) for the oxytocin subsample.

	Females (N=94)		Males (N=76)				
	CD (N=45)	HCs (N=49)	CD (N=35)	HCs (N=41)	Group	Sex	Group x sex
	Mean (SD) or N (%)				*p*	*p*	*p*
Age (years)	14.51 (1.5)	14.53 (2.01)	14.34 (2.1)	14.59 (2.00)	.66	.85	.70
Estimated Full-Scale IQ	95.02 (11.9)	101.53 (12.6)	102.43 (12.6)	108.37 (12-0)	<.01	<.001	.88
Parental educational status	3.06 (1.1)	3.79 (1.1)	3.34 (0.7)	3.74 (1.0)	<.01	.46	.30
Body Mass Index	24.30 (8.0)	20.31 (4.1)	21.78 (4.2)	20.48 (3.5)	<.01	.15	.10
Start time Trier Social Stress Test (hh:mm)	15:04 (00:49)	15:08 (00:33)	15:24 (00:30)	14:59 (00:42)	.09	.39	.02
Pubertal Status					.28	<.001	
Early-pubertal	0 (0.0)	1 (2.0)	8 (22.9)	12 (29.3)			
Mid-pubertal	3 (6.7)	9 (18.4)	9 (25.7)	11 (26.8)			
Late-pubertal	33 (73.3)	27 (55.1)	18 (51.4)	17 (41.5)			
Post-pubertal	9 (20.0)	12 (24.5)	0 (0.0)	1 (2.4)			
Current smoking	25 (55.6)	1 (2.0)	18 (51.4)	7 (17.1)	<.001	.46	
Any medication[a]	28 (62.2)	7 (14.3)	9 (25.7)	2 (4.9)	<.001	<.01	
Neuroleptics	4 (8.9)	0 (0.0)	3 (8.6)	0 (0.0)	<.01	.92	
Stimulants	8 (17.8)	0 (0.0)	5 (14.3)	0 (0.0)	<.001	.64	
Non-stimulants	0 (0.0)	0 (0.0)	1 (2.9)	0 (0.0)	.29	.27	
SSRIs/antidepressants	4 (8.9)	0 (0.0)	2 (5.7)	0 (0.0)	<.01	.57	
Contraceptives	18 (53.8)	5 (14.3)	0 (0.0)	0 (0.0)	<.01		
Other	7 (15.6)	7 (6.0)	1 (2.9)	2 (4.9)	.28	.10	
Comorbidities							
Lifetime ADHD	16 (35.6)	0 (0.0)	22 (62.9)	0 (0.0)	<.001	.06	
Lifetime substance use disorder	10 (22.2)	0 (0.0)	11 (31.4)	1 (2.4)	<.001	.32	
Lifetime anxiety disorder[b]	11 (24.4)	2 (4.1)	15 (42.9)	2 (4.9)	<.001	.15	
Lifetime depressive disorder	21 (46.7)	2 (4.1)	8 (22.9)	0 (0.0)	<.001	.02	
Lifetime PTSD	8 (17.8)	0 (0.0)	5 (14.3)	1 (2.4)	<.001	.89	
Pre- and postnatal risk factors							
Prenatal smoking	12 (36.4)	6 (14.6)	14 (46.7)	4 (10.3)	<.001	.81	
Prenatal violence	11 (30.6)	3 (7.3)	6 (20.7)	2 (5.1)	<.01	.28	
Adverse family situation					<.001	.65	
Either disharmony or isolation	10 (29.4)	7 (17.1)	8 (28.6)	11 (28.9)			
Both disharmony and isolation	12 (35.3)	1 (2.4)	8 (28.6)	1 (2.6)			
Number of traumatic events experienced	2.80 (1.9)	1.14 (1.2)	1.94 (1.7)	1.37 (1.2)	<.001	.18	.02

Note. [a]"Non-stimulants" include atomoxetine, "Other" medication includes for example asthma medication, painkiller, or vitamin preparation. Participants may have reported use of more than one medication category, thus the numbers do not sum up to 100%. [b]Rates of lifetime anxiety

disorder cover lifetime panic disorder, separation anxiety disorder, avoidant disorder, simple phobia, social phobia, agoraphobia, overanxious disorder or generalized anxiety disorder.

Table S3. Psychoneuroendocrinological stress measures as mediators of environmental risk and comorbid psychiatric disorders on Conduct Disorder.

	Direct effect (X→Y)	*p*	Direct effect (X→M)	*p*	Direct effect (M→Y)	*p*	Indirect effect (X→M→Y)	95% CI [LB; UB]	Total effect	*p*	Proportion of total effect mediated [%]
AUC_I											
Cortisol											
Prenatal smoking	1.43	<.001	-1.53	.29	-0.08	<.01	0.12	[-0.10; 0.37]	1.54	<.001	7.64
Prenatal violence	1.72	<.01	-2.88	.15	-0.07	<.01	0.19	[0.01; 0.51]	1.91	<.01	9.80
Adverse family situation	1.46	<.001	-1.88	.04	-0.06	<.01	0.12	[0.02; 0.27]	1.57	<.001	7.42
Trauma exposure	0.45	<.001	-0.30	.42	-0.08	<.001	0.02	[-0.03; 0.09]	0.47	<.001	5.12
Testosterone											
Prenatal smoking	1.51	<.001	0.52	.83	-0.04	<.01	-0.02	[-0.21; 0.16]	1.49	<.001	1.27
Prenatal violence	1.82	<.01	-0.70	.83	-0.04	<.01	0.03	[-0.20; 0.21]	1.85	<.01	1.35
Adverse family situation	1.49	<.001	-2.06	.19	-0.03	.06	0.05	[-0.02; 0.17]	1.54	<.001	3.43
Trauma exposure	0.45	<.001	-0.12	.85	-0.04	<.01	0.004	[-0.05; 0.06]	0.45	<.001	0.92
Oxytocin											
Prenatal smoking	1.53	<.01	-0.29	.10	-0.44	.19	0.13	[-0.01; 0.47]	1.65	<.001	7.70
Prenatal violence	1.91	<.01	-0.20	.35	-0.39	.20	0.08	[-0.08; 0.40]	1.98	<.01	3.86
Adverse family situation	1.33	<.001	-0.05	.63	-0.40	.21	0.02	[-0.07; 0.19]	1.36	<.001	1.61
Trauma exposure	0.38	.01	0.00	.98	-0.38	.17	0.001	[-0.05; 0.04]	0.38	<.001	0.26
Psychological stress											
Prenatal smoking	1.49	<.001	0.58	.67	0.06	<.001	0.03	[-0.12; 0.21]	1.52	<.001	2.10
Prenatal violence	1.64	<.001	4.05	.03	0.05	<.01	0.19	[-0.03; 0.55]	1.83	<.001	10.57
Adverse family situation	1.50	<.001	2.07	.01	0.04	.02	0.09	[0.004; 0.23]	1.59	<.001	5.41
Trauma exposure	0.54	<.001	1.20	<.001	0.03	.07	0.03	[-0.01; 0.08]	0.57	<.001	5.15
INCREASE											
Cortisol											
Prenatal smoking	1.40	<.001	-0.82	.16	-0.26	<.001	0.22	[-0.03; 0.58]	1.61	<.001	13.42
Prenatal violence	1.66	<.01	-1.50	.06	-0.19	<.001	0.28	[0.04; 0.83]	1.94	<.01	14.47
Adverse family situation	1.42	<.001	-1.02	<.01	-0.21	<.01	0.22	[0.08; 0.51]	1.64	<.001	13.23
Trauma exposure	0.41	<.001	-0.24	.11	-0.23	<.001	0.06	[-0.01; 0.15]	0.47	<.001	11.85
Testosterone											
Prenatal smoking	1.52	<.001	0.38	.82	-0.06	<.01	-0.02	[-0.24; 0.19]	1.50	<.001	1.56
Prenatal violence	1.74	<.01	-1.73	.44	-0.06	<.01	0.10	[-0.10; 0.36]	1.84	<.01	5.43
Adverse family situation	1.49	<.001	-1.55	.14	-0.05	.01	0.07	[-0.01; 0.24]	1.57	<.001	4.79
Trauma exposure	0.43	<.001	-0.52	.21	-0.06	<.01	0.03	[-0.02; 0.09]	0.46	<.001	6.85
Oxytocin											
Prenatal smoking	1.47	<.01	-0.27	.06	-0.84	.11	0.22	[0.03; 0.78]	1.69	<.001	13.19
Prenatal violence	1.84	<.01	<0.01	.18	-0.82	.10	0.19	[-0.02; 0.73]	2.03	<.01	9.16

	Direct effect (X→Y)	*p*	Direct effect (X→M)	*p*	Direct effect (M→Y)	*p*	Indirect effect (X→M→Y)	95% CI [LB; UB]	Total effect	*p*	Proportion of total effect mediated [%]
INCREASE											
Oxytocin											
Adverse family situation	1.31	<.001	-0.08	.35	-0.78	.13	0.06	[-0.08; 0.30]	1.38	<.001	4.69
Trauma exposure	0.38	.01	-0.02	.54	-0.80	.08	0.02	[-0.05; 0.10]	0.39	<.001	4.47
Psychological stress											
Prenatal smoking	1.44	<.001	0.13	.77	0.04	.34	0.01	[-0.05; 0.07]	1.45	<.001	0.69
Prenatal violence	1.70	<.001	0.43	.47	0.03	.50	0.01	[-0.08; 0.13]	1.71	<.001	0.58
Adverse family situation	1.53	<.001	-0.07	.81	0.02	.69	0.00	[-0.04; 0.03]	1.53	<.001	0.07
Trauma exposure	0.56	<.001	0.11	.33	0.01	.87	0.00	[-0.02; 0.02]	0.56	<.001	0.18
RECOVERY											
Cortisol											
Prenatal smoking	1.33	<.01	-0.79	.08	-0.33	<.001	0.26	[0.02; 0.64]	1.59	<.001	16.32
Prenatal violence	1.69	<.01	-1.08	.09	-0.22	<.01	0.24	[-0.0004; 0.86]	1.93	<.01	12.41
Adverse family situation	1.40	<.001	-0.84	<.01	-0.28	<.01	0.23	[0.12; 0.49]	1.64	<.001	14.26
Trauma exposure	0.39	<.001	-0.24	<.05	-0.26	<.001	0.06	[0.01; 0.15]	0.45	<.001	13.40
Testosterone											
Prenatal smoking	1.43	<.001	0.09	.96	-0.02	.13	0.00	[-0.11; 0.09]	1.42	<.001	-0.14
Prenatal violence	1.81	<.01	-3.79	.15	-0.02	.23	0.06	[-0.02; 0.21]	1.88	<.01	3.42
Adverse family situation	1.54	<.001	-0.52	.68	-0.03	.08	0.01	[-0.05; 0.08]	1.55	<.001	0.85
Trauma exposure	0.39	<.001	-1.33	<.01	-0.02	.13	0.03	[-0.01; 0.07]	0.41	<.001	6.13
Oxytocin											
Prenatal smoking	1.53	<.01	-0.21	.11	-0.83	.07	0.18	[0.02; 0.74]	1.70	<.001	10.37
Prenatal violence	1.79	<.01	-0.28	.08	-0.93	.04	0.26	[0.03; 0.80]	2.05	<.01	12.83
Adverse family situation	1.28	<.001	-0.14	.09	-0.90	.06	0.13	[-0.01; 0.43]	1.41	<.001	8.99
Trauma exposure	0.33	.03	-0.06	.06	-1.03	.03	0.07	[-0.001; 0.20]	0.40	<.001	16.47
Psychological stress											
Prenatal smoking	1.43	<.001	0.45	.31	0.10	.04	0.04	[-0.04; 0.18]	1.47	<.001	2.72
Prenatal violence	1.64	<.001	1.15	.06	0.07	.11	0.08	[-0.02; 0.27]	1.72	<.001	4.65
Adverse family situation	1.51	<.001	0.60	.03	0.05	.34	0.03	[-0.03; 0.11]	1.54	<.001	1.95
Trauma exposure	0.55	<.001	0.20	.08	0.05	.22	0.01	[-0.01; 0.04]	0.56	<.001	1.79

Note. [a]The standardized estimates of the effects are reported. Significant mediation effects are highlighted in bold. AUC_I=Area under the curve with respect to increase, M=psychoneuroendocrine mediator, UB=upper bound, X=risk factor, Y=CD status.