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Running title: Basal neuroendocrinology of Conduct Disorder

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Abstract

Conduct Disorder (CD) is characterized by severe aggressive and antisocial behaviour. The stress hormone system has frequently been investigated as a neurobiological correlate of CD, while other interacting neuroendocrine biomarkers of sex hormone or neuropeptide systems have rarely been studied, especially in females. We examined multiple basal neuroendocrine biomarkers in female and male adolescents with CD compared to healthy controls (HCs), and explored whether they mediate effects of environmental risk factors on CD. Within the FemNAT-CD study, salivary cortisol, alpha-amylase, testosterone, dehydroepiandrosterone-sulfate (DHEA-S), estradiol, progesterone, oxytocin, and arginine-vasopressin were measured under basal conditions in 166 pubertal adolescents with CD, and 194 sex-, age-, and puberty-matched HCs (60% females, 9-18 years). Further, environmental risk factors were assessed. Single hormone analyses showed higher DHEA-S, and lower estradiol and progesterone levels in both females and males with CD relative to HCs. When accounting for interactions between neuroendocrine systems, a male-specific sex hormone factor (testosterone/DHEA-S) predicted male CD, while estradiol and a stress-system factor (cortisol/alpha-amylase) interacting with oxytocin predicted female CD. Estradiol, progesterone, and oxytocin partly explained associations between early environmental risk and CD. Findings provide evidence for sex-specific associations between basal neuroendocrine measures and CD. Especially altered sex hormones (androgen increases in males, estrogen reductions in females) robustly related to CD, while basal stress-system measures did not. Early environmental risk factors for CD may act partly through their effects on the neuroendocrine system, especially in females. Limitations (e.g., basal neuroendocrine assessment, different sample sizes per sex, pubertal participants, exploratory mediation analyses) are discussed.

1. Introduction

Conduct Disorder (CD) is a psychiatric disorder of childhood and adolescence characterized by persistent aggressive and antisocial behaviour (American Psychiatric Association, 2000). It has a highly negative impact on the affected individuals, their families and society in general (Erskine et al., 2014; Rivenbark et al., 2018). Yet, it is one of the most understudied mental disorders (Fairchild et al., 2019). CD occurs more frequently in males (2-5%) than females (1-3%) with increasing prevalence in adolescence, especially in females (Freitag et al., 2018). However, most studies on CD focus on males. CD shows a complex aetiology based on an interaction of genetic, neurobiological and psychosocial risk factors (Fairchild et al., 2019). For several decades, numerous environmental risks have been considered as major contributing factors for CD of which prenatal, perinatal and familial factors (e.g., maltreatment and trauma exposure) are the most widely replicated (Bernhard et al., 2018a). More recently, various neurobiological alterations (e.g., differences in brain structure and function, attenuated autonomic nervous system (ANS) functioning) have been reported in CD (Freitag et al., 2018). Given the assumed interplay of environmental and neurobiological risk factors, particularly early life stressors may induce lasting neurobiological changes, thereby increasing risk for CD (Bunea et al., 2017; Cushing and Kramer, 2005).

To gain more knowledge about possible stress-related neurobiological changes in CD, a closer consideration of the underlying neuroendocrinology is essential. Generally, the body's regulation of stress is managed by the hypothalamic-pituitary-adrenal (HPA)-axis and ANS (Gunnar and Quevedo, 2007). Salivary cortisol is a commonly used peripheral biomarker of HPA-axis functioning (Kudielka et al., 2012), while salivary alpha-amylase was proposed as a peripheral biomarker of sympathetic ANS activity (Nater and Rohleder, 2009). The HPA-axis and ANS reciprocally interact during acute and chronic stress (Bauer et al., 2002). HPA-axis functioning is also influenced by hormones of the hypothalamic-pituitary-

gonadal (HPG)-axis (Oyola and Handa, 2017), such as testosterone, dehydroepiandrosterone-sulfate (DHEA-S), estradiol, and progesterone. These sex hormones belong to the same class of steroid hormones as cortisol, which can pass the blood-brain barrier (Gunnar and Quevedo, 2007). Furthermore, the neuropeptide system influences the stress hormone system with antagonistic effects of oxytocin (stress-buffering) and arginine-vasopressin (stress-inducing) on HPA-axis activity (Jurek and Neumann, 2018). While neither of these neuropeptides can pass the blood-brain-barrier, positive correlations between salivary (peripheral) and central neuropeptide levels were reported (Martin et al., 2018). Neuropeptides and sex hormones also interact, with estradiol stimulating oxytocin-producing neurons and testosterone influencing the production and neural activity of arginine-vasopressin (Bos et al., 2012). Overall, stress regulation is influenced by a complex, mutually interacting set of neuroendocrine systems. Thus, a multiple system approach is needed when exploring the underlying neuroendocrinology of psychiatric disorders such as CD.

Yet, previous neuroendocrinological studies in CD, predominantly conducted in males, typically focused on either HPA-axis, ANS-functioning or male sex hormones in isolation (Freitag et al., 2018). Consequently, there has been little research testing for interaction effects between hormones or/and neuropeptides in CD. Regarding the HPA-axis, reduced basal salivary cortisol in females and males with CD compared to healthy individuals have been reported and integrated into the “fearlessness” or “low-arousal” theory of CD (Cappadocia et al., 2009). More recent studies reporting similar or even increased basal cortisol levels in CD contradict these theories (Feilhauer et al., 2013; Northover et al., 2016; Young et al., 2012). Considering an alternative measure of stress functioning, lower basal salivary alpha-amylase was reported in males (Angyal et al., 2016), but has not been studied in females with CD. Regarding the HPG-axis, positive correlations between the male sex hormone testosterone (Yildirim and Derksen, 2012) or its precursor DHEA-S (Soma et al.,

2015) and aggression have often been reported, particularly in males, although findings have also been inconsistent (Constantino et al., 1993; Dmitrieva et al., 2001; Dorn et al., 2009). Earlier results showed that testosterone interacted with cortisol to predict aggression, but a recent meta-analysis did not support this (Dekkers et al., 2019). Considering female sex hormones, only one study examined basal estradiol, reporting no case-control-differences in females with CD (Pajer et al., 2006). Other sex hormones have not yet been investigated in CD. Regarding neuropeptides, basal salivary oxytocin correlated negatively with disruptive behaviour disorders and conduct problem severity in male adolescents (Bakker-Huvenaars et al., 2020; Levy et al., 2015). In females with CD, oxytocin has not been studied. Arginine-vasopressin has not been studied at all in CD, despite its well-established role in regulating aggressive behaviour in animals and humans (Jurek and Neumann, 2018). Consequently, in the present study we set out to investigate multiple steroid hormones and neuropeptides in the same adolescent sample, testing for sex-specific associations with, and interactions between, these biomarkers and CD.

Given the impact of early life stress (e.g., Cushing and Kramer, 2005), associations between neuroendocrine alterations and particularly early prenatal or perinatal environmental risk factors may be relevant for later psychopathology. Early-life adversities such as maternal smoking in pregnancy, neglect, parental conflicts, parental psychopathology, and exposure to maltreatment and trauma have been well replicated in CD (Bernhard et al., 2018a), and are associated with altered cortisol (Koss and Gunnar, 2018), testosterone (Yildirim and Derksen, 2012) or oxytocin (Tobon et al., 2018). A current review highlighted the importance of considering the impact of trauma in neuroendocrine research on adolescent aggression (Fragkaki et al., 2018). Given that early adversity leads to long-term epigenetic changes related to neuroendocrine functioning and aggressive behaviour (Chistiakov and Chekhonin, 2019; Cushing and Kramer, 2005), research exploring the potential mediating role of

associated hormones and neuropeptides as an underlying mechanism of early environmental risk in CD is warranted.

To address the above-mentioned research gaps in CD, this study adopted an integrated approach by exploring basal neuroendocrinological alterations in the HPA-axis (cortisol, alpha-amylase), HPG-axis (testosterone, DHEA-S, estradiol, progesterone) and neuropeptide (oxytocin, arginine-vasopressin) systems in females and males with CD compared to age-, sex- and puberty-matched healthy adolescents. In addition, neuroendocrine markers are explored as mediators of early environmental risk factors for CD. As evidence in this area is inconsistent and limited, especially in females with CD, our study is primarily exploratory in nature. Very few studies in this field have investigated more than a single hormone at once, and little is known about sex differences in the relationship between neuroendocrine alterations and CD or about neuroendocrine mediation of early environmental risk factor effects. Thus, using an exploratory approach accounting for interactions between neuroendocrine systems and relevant confounding factors, we aim to generate evidence that will provide a platform for future studies.

2. Experimental procedures

Participants

This study included 166 adolescents with CD (99 females) and 194 healthy adolescents (117 females) aged 9-18 years from the FemNAT-CD European multi-site study ("Neurobiology and Treatment of Female Conduct Disorder") study (Freitag et al., 2018) (for site distribution see Supplementary Table S1). Local ethical committees at each site approved the study. Participants were recruited from clinics, schools, and the community. Written informed consent was obtained from all participants or their parents/caregivers prior to participation. Exclusion criteria included IQ<70, pre-pubertal status, contraceptive use, pregnancy, last menstruation >six months ago, history of neurological disorder, traumatic brain injury, schizophrenia, autism spectrum disorder, or current mania or bipolar disorder. Adolescents with CD met current CD diagnosis according to DSM-IV-TR (American Psychiatric Association, 2000). Healthy controls (HCs) had no current DSM-IV-TR disorder nor a history of disruptive behaviour- or attention-deficit/hyperactivity disorder (ADHD).

Current and lifetime psychiatric disorders were assessed using the Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime version (K-SADS-PL), and IQ using the Wechsler Intelligence Scales, as described recently (Kohls et al., 2020; see also online Supplement). Pubertal status was assessed using the Pubertal Development Scale (Petersen et al., 1988), a five-level categorical (pre-/early-/mid-/late-/post-pubertal) self-report measure (Cronbach's α=0.77) on pubertal growth (e.g. changes in body hair, voice or breast development) with four response options (not yet started, barely started, definitely started, seems complete). Given the associations between early life stress and neuroendocrine alterations, prenatal, perinatal and familial early environmental risk factors (see online Supplement) were measured via a structured medical history interview (Freitag et al., 2012)

conducted with the parents/caregivers: prenatal smoking and violence exposure, early maternal age at birth, single parenthood (single mother or father) and early insufficient care (frequent change of caregivers) during participant's first year of life, adverse family situation (familial disharmony and isolation), low parental educational status, and parental psychopathology. Furthermore, from the K-SADS-PL PTSD section the number of traumatic events experienced was included as environmental risk factor.

Neuroendocrinological assessment and quantification

Saliva samples were collected during the afternoon (between 13:00-18:00h) after one hour resting period to minimize effects of earlier physical exercise or stressful events. Neither substance/alcohol use on the day of assessment nor drinking, eating, or smoking during resting and assessment period were permitted (controlled by the presence of the test leader). To analyse all respective neuroendocrine measures, two saliva samples per participant were collected. For analyses of steroid hormones and alpha-amylase, saliva was collected using a 2 ml cryogenic vial (VWR International, Darmstadt) plus a saliva collection aid (Salimetrics, LLC, USA), into which participants spat until ~1.5 ml saliva was collected. For neuropeptide analyses, participants were instructed to gently chew on the cotton swab of a Salivette® (Sarstedt, Nümbrecht, Germany) for 60 seconds. To ensure comparability across sites, manuals of standardized operating procedures were used at each site during all assessments with adherence controlled by external monitoring. All saliva samples were stored at -20°C until analysis. Saliva samples were analysed by two laboratories: all cryogenic vials for determination of free salivary cortisol (nmol/l), alpha-amylase (U/ml), testosterone, DHEA-S, estradiol, and progesterone (all pg/ml) were analysed by Daacro (Trier, Germany) employing an enzyme immunoassay kit or a kinetic enzyme assay kit for alpha-amylase (Salimetrics, LLC, USA) formatted to minimize cross-reactivity for related steroids. The corresponding inter-assay coefficients of variation were in the commonly accepted range (below 15%)

(Salimetrics, 2021). All Salivettes® for determination of oxytocin and arginine-vasopressin (pg/ml) concentrations were analysed by RIAgnosis (Sinzing, Germany) employing a radioimmunoassay as previously described (de Jong et al., 2015). For each sample 300 µl of saliva was evaporated (Concentrator, Eppendorf, Germany) and 50 µl of assay buffer was added followed by 50 µl rabbit antibody against oxytocin and arginine-vasopressin, respectively. The inter-assay coefficients of variation were <10%.

Statistical Analyses

For this study, a subsample of participants from the FemNAT-CD study was included after individual 1:1 case-control matching, with exact matching for sex and pubertal status, and range matching for age (±1) using PROC SQL procedure in SAS® (v 9.4, SAS Inc., Cary/NC, USA). All participants were tested between March 2014-July 2016. After matching, some individuals had to be excluded (e.g., due to missing neuroendocrine data) resulting in the final presented sample.

The groups' demographic and clinical characteristics were compared using two-sample t-/U- or chi-squared tests. Neuroendocrinological measures were transformed by natural logarithmic transformation for normal distribution and Z-standardized for comparison (for untransformed data, see Supplementary Table S2). All analyses were controlled for current smoking, body mass index (BMI), age, time of saliva sampling, and assessment site due to significant group differences or predictive effects for neuroendocrine measures. Medication use did not predict any neuroendocrine measure therefore this was not included as a covariate. Pubertal status correlated highly with age (r_s=.68, p<.001), was matched between groups, and therefore not included as an additional covariate.

First, to explore effects of group, sex and group-by-sex interactions on all neuroendocrine markers without considering interactions between neuroendocrine systems, univariate analyses of covariance (ANCOVA) were run with group (CD vs. HCs) and sex

(female vs. male) as between-subject factors. Second, to simultaneously explore all neuroendocrine measures, a multivariable logistic regression model was applied with the dependent variable group status (CD vs. HCs) and all neuroendocrine markers as independent variables. Analyses were performed separately for both sexes due to neuroendocrinological differences during puberty and different sample sizes for females and males. Third, to explore neuroendocrine interaction effects on CD status, a sex-specific data reduction of neuroendocrine measures was done by principal component analysis. Components with Eigenvalue >1 (Kaiser-Guttmann criterion) were extracted and varimax rotation applied. Individual factor loadings were calculated as the weighted sum of the respective neuroendocrine measures. Another multivariable logistic regression model was applied separately in females and males to explore main and two-way interaction effects of the above identified neuroendocrine factors on group status.

Lastly, to explore if the relation between an environmental risk factor and CD was mediated by any of the neuroendocrine measures, separate mediation analyses were performed in females and males. For each risk factor and possible mediator (neuroendocrine measures with main or interaction effects on risk for CD), an exploratory mediation analysis was run yielding direct, indirect, and total effects: a) a linear regression model of the mediator as a function of the risk factor, b) a logistic regression model of the case-control-status as a function of the mediator and the risk factor, and c) a logistic regression model of the case-control-status as a function of the risk factor. The standardized estimates of the effects are reported together with the proportion of the total effect mediated by the neuroendocrine measure. Applying bootstrapping with 1000 resamples, percentile bootstrap confidence intervals (CI) for the indirect effect were computed. Bootstrapping included repeatedly nonparametric sampling from the data set and estimation of the indirect effect in each resampled dataset resulting in an empirical distribution of the indirect effect. The null

hypothesis of no mediation effect was rejected if $0 \notin 95\%$ CI (Preacher and Hayes, 2008). As several risk factors occurred infrequently (especially in HCs), reducing the power to detect a true effect, and the mediation analysis performed in an exploratory fashion, only neuroendocrine markers explaining >10% of the total risk effect were considered. Given the exploratory design of the mediation analyses to investigate possible associations of neuroendocrinological measures and environmental risk factors, the presentation of results is rather descriptive, thus no correction for multiple comparisons was applied.

Effect sizes were expressed as Cohen's *d* with 0.2, 0.5, and 0.8 representing small, medium and large effects, respectively (Cohen, 1988), or odds ratio (OR) with ORs below/above 1 indicating relevant effects. Our sample size enabled us to detect even small effects with Cohen's *ds* of 0.3 or above, including group-by-sex interactions, or an OR>=1.52 or OR<=0.66 in females, and an OR>=1.69 or OR<=0.59 in males, with a power of 80% and a two-sided significance level of 5%, assuming R^2=0.1 for confounding factors (G*Power 3.1.9.2).

3. Results

Table 1 presents descriptive statistics for demographic and clinical characteristics, environmental risk factors, and psychiatric comorbidities. Groups did not significantly differ in sex, age, or pubertal status. Rates of current smoking and medication use (see Supplementary Table S3) were higher, while IQ was lower in CD than HC participants. Groups differed for all assessed risk factors and psychiatric comorbidities with higher rates in CD than HCs. Most female participants had started menstruating (CD 93%, HCs 94%) with comparable days since the last menstruation [Mean (SD): CD 15.07 (10.12), HCs 14.56 (9.32), p=.73].

[Table 1]

Univariate analyses of covariance

ANCOVAs revealed effects of group for DHEA-S [F(1,327)=5.91, p=.02, d=0.27], estradiol [F(1,323)=4.97, p=.03, d=-0.24], and progesterone [F(1,324)=5.28, p=.02, d=-0.25]. Participants with CD showed higher DHEA-S, but lower estradiol and progesterone levels than HCs. No group effects were found for cortisol [F(1,327)=0.03, p=.87, d=0.02], alpha-amylase [F(1,327)=0.03, p=.86, d=0.02], testosterone [F(1,326)=.12, p=.73, d=0.04], oxytocin [F(1,326)=0.24, p=.62, d=0.05], and arginine-vasopressin [F(1,327)=0.18, p=.67, d=0.05]. Testosterone was higher in males overall than females [F(1,233)=111.91, p<.001, d=1.18]. No other sex effects or group-by-sex interactions were detected (Figure 1; Supplementary Table S4).

[Figure 1]

Multivariable logistic regression analyses

Table 2 provides ORs for main effects of neuroendocrine measures predicting CD status in females and males, respectively. In females, estradiol showed an OR below 1 (OR 0.57, 95%

CI 0.34-0.97, *p*=.04), indicating low estradiol is a risk factor for CD. In males, no significant main effects emerged.

[Table 2]

Multivariable logistic regression analysis of the factors emerging from the principal component analysis

Sex-specific data reduction of the neuroendocrine measures resulted in four different factor structures in both sexes (see Table 3). In females, Factor 1 loaded highly on testosterone, progesterone and estradiol, Factor 2 on DHEA-S and arginine-vasopressin, Factor 3 on cortisol and alpha-amylase, and Factor 4 on oxytocin. In males, Factor 1 loaded highly on progesterone and estradiol, Factor 2 on testosterone and DHEA-S, Factor 3 on alpha-amylase and arginine-vasopressin, and Factor 4 on oxytocin. Cortisol did not load onto a specific factor in males, but loaded equally highly onto Factors 1 and 2, and equally low onto Factors 3 and 4.

[Table 3]

In females, there were no main effects of any of the factors, but an interaction between Factors 3 and 4 (OR 0.52, 95% CI 0.32-0.86, *p*=.01) emerged as a predictor of CD. Risk for CD was increased in females with low levels of cortisol/alpha-amylase (Factor 3) and high oxytocin (Factor 4), or the opposite relationship, i.e., high cortisol/alpha-amylase and low oxytocin. Risk for CD was decreased if both Factors were either low or high, suggesting coordinated activation across these systems (see Figure 2).

In males, a main effect of Factor 2 (OR 2.24, 95% CI 1.16-4.31, *p*=.02) indicated that elevated testosterone and DHEA-S predicted CD, while no interaction effects between factors emerged.

[Figure 2]

Mediation analyses

Several environmental risk factors showed a direct effect on CD (see Supplementary Table S5). We therefore tested whether these associations were mediated by neuroendocrine measures. No indirect effects of the assessed neuroendocrine measures mediating the correlation between risk factors and CD status emerged. Nevertheless, alterations in estradiol explained 25% of the total risk effect of early insufficient care and 17% of the risk effect of single parenthood on CD in females, and 12% of the risk effect of parental psychopathology on CD in males. In females only, changes in progesterone explained 15% of the total risk effect of early insufficient care and 10% of the risk of single parenthood, while alterations in oxytocin explained 12% of the risk of early insufficient care on CD. None of the other studied neuroendocrine markers explained >10% of the total risk effect between the risk factor in question and CD.

4. Discussion

To our knowledge, this is the largest, and most comprehensive, study on the basal neuroendocrinological systems in CD, and the first to simultaneously investigate multiple basal biomarkers of the HPA-axis (cortisol, alpha-amylase), HPG-axis (testosterone, DHEA-S, estradiol, progesterone), and neuropeptide (oxytocin, arginine-vasopressin) systems. We compared females and males with CD and sex-, age- and puberty-matched HCs to explore potential sex-specific neuroendocrine alterations in CD. In addition, we explored whether neuroendocrine biomarkers mediate the effects of environmental risk factors on CD. Our results challenge earlier notions of attenuated basal stress system functioning in CD ('low arousal' theories), as we found no reliable differences in the basal stress hormone system between CD and HCs. Further, our findings considerably extend previous work by providing evidence for basal alterations in the sex hormone system in both females and males with CD compared to HCs. Only in females with CD, dysregulation of the oxytocin neuropeptide system in interaction with stress system measures was identified. There was also preliminary evidence that sex hormone and neuropeptide disturbances partly mediate the relationship between early life adversity and CD. Overall, our study significantly enhances our understanding of the sex-specific neurobiology of CD. Following the order of the HPA-axis, the HPG-axis, and the neuropeptide system, considering main and interaction effects, we now discuss our findings in more detail, identifying new areas for future research in CD.

Previously, *stress hormone system* alterations have been a major focus of neuroendocrine research in CD (Cappadocia et al., 2009). Accounting for neuroendocrine interactions and confounding effects, our analyses provided no evidence for altered HPA-axis and ANS biomarkers in either females or males, as assessed using basal salivary cortisol and alpha-amylase. While earlier studies reported attenuated basal cortisol levels - particularly in

males with CD - evidence for basal HPA-axis alterations in CD has been questioned more recently (Fairchild et al., 2018). Our results indicate basal markers of HPA-axis and ANS-functioning are not robustly associated with CD in female or male adolescents. This is in line with other findings from the FemNAT-CD project reporting no associations between basal ANS activity and adolescent CD using peripheral psychophysiological methods in N=1010 youths (partly including the current subsample and additional participants from FemNAT-CD; Oldenhof et al., 2018; Prätzlich et al., 2019). Considering relevant neuroendocrine confounders seems to be essential for future work. Further, HPA-axis alterations in CD may be more pronounced in response to stress, where hypo-reactivity is typically observed (Fairchild et al., 2018). It might be more informative to investigate stress hormone system measures in CD under stressful rather than basal conditions, especially as research on females with CD is missing here too (Freitag et al., 2018). Considering previously overlooked neuroendocrine interactions, we found an interaction effect of stress system measures and oxytocin in females only (see below).

Contrary to our findings for the stress hormone system, our analyses indicate *sex hormone system* alterations in both females and males with CD. Regarding male sex hormones, simple group comparisons revealed no differences in testosterone, but rather in its precursor DHEA-S, with higher levels in CD participants of both sexes compared to HCs. Yet, results of single group comparisons are limited: while major - previously neglected - confounder were controlled for, the influence of other neuroendocrine measures was not. Accordingly, when considering neuroendocrine interactions, a combined factor of testosterone and DHEA-S was positively associated with 2.2 times greater odds for CD in males only. These results fit with reports of increased testosterone (Yildirim and Derksen, 2012) and DHEA-S (Soma et al., 2015) in males with CD. In our sample DHEA-S and testosterone strongly correlated in males with CD (r=.54, p<.001, N=67), but only weakly in male controls

(r=.29, p<.01, N=77; all controlled for age). Results indicate a combined factor of testosterone and DHEA-S to be more predictive of CD in males than the singular hormones itself, which may explain previous inconsistent results. Further studies should replicate findings and focus on the joint effects of DHEA-S and testosterone for CD. When accounting for neuroendocrine interactions, the group effect for DHEA-S was abolished in females, indicating other sex hormones to be more powerful (see below). No interaction between testosterone and cortisol was observed. Thus, our data does not support the dual-hormone hypothesis, consistent with recent meta-analytic evidence (Dekkers et al., 2019).

Regarding female sex hormones, simple group comparisons showed lower salivary estradiol levels in both females and males with CD than in HCs. Accounting for neuroendocrine interactions, significant effects of estradiol were only observed in females. Females with decreased estradiol levels showed 40% higher odds for CD. Thus, low estradiol appeared to be a risk factor for CD in females, while higher estradiol was protective. However, ORs for estradiol were similar in females and males. The smaller sample size in males might have reduced statistical power. Only one previous study assessed plasma estradiol in a small sample of adolescent females with CD reporting no differences between CD and HC participants (Pajer et al., 2006), likely due to low statistical power. Taking a broader perspective, estradiol has been suggested to play a neuroprotective role against other psychiatric disorders characterized by social deficits such as autism spectrum disorder (Crider and Pillai, 2016), and has been described as modulator of neural processes underlying social behaviour and aggression (Montoya and Bos, 2017). Furthermore, both females and males with CD showed lower progesterone levels than sex-matched HCs in simple group comparisons. When accounting for neuroendocrine interactions, this group effect disappeared. Progesterone is a precursor for mainly estrogen, but also androgen, such as testosterone, distribution. Thus, when considering neuroendocrine interactions, the observed effects of

estradiol, testosterone and DHEA-S may swallow progesterone effects on CD risk. As this is the first study investigating salivary estradiol and progesterone levels in females and males with CD, with widespread observed alterations in both hormones, our results strongly suggest further research on sex hormones in CD is merited.

Taken together, sex-specific findings of sex hormone alterations in CD point towards a strong role of puberty-related neuroendocrine changes in CD. This is especially relevant given the reported increasing prevalence of CD in puberty (Maughan et al., 2004) and the fact, that early puberty has been described as a risk factor for CD, especially in females (Burt et al., 2006). As both androgens and estrogens target brain regions involved in emotion regulation and impulse control (e.g. the amygdala) (Nguyen et al., 2016; Spiteri et al., 2010), which have been shown to be altered in CD (Rogers and De Brito, 2016), dysregulation of the sex hormone system may underlie CD-related behavioural symptoms supporting aggressive, impulsive and risk-taking tendencies. Longitudinal studies in children, adolescents, and adults with a history of CD should clarify if sex hormones are specific neuroendocrine risk factors for CD, and if stabilization or normalization of sex hormone levels over time is related to persistence or desistence of CD. Further, investigating the underlying neurobiological mechanisms, and understanding interactions between neuroendocrine measures and brain function in adolescent CD is an important topic for future research.

For the *neuropeptide system*, no main effects of oxytocin and arginine-vasopressin were found. Oxytocin interacted negatively with the cortisol/alpha-amylase stress system factor to influence risk for female CD. High oxytocin levels and low stress system measures (and vice versa) were associated with 50% higher odds for CD in females. CD risk was lowest in females with parallel, and presumably more coordinated, HPA-axis/ANS and oxytocin activation (either low or high across systems). Oxytocin has been described as being co-activated with cortisol during acute psychosocial stress in healthy adolescents (Bernhard et

al., 2018b) and adults (de Jong et al., 2015). Although basal rather than reactive neuroendocrine stress system markers and oxytocin were studied here, results point towards a differential association of HPA-axis, ANS and oxytocin in females with CD. As this is the first study investigating oxytocin and alpha-amylase in females with CD, and the first to consider interactions between these systems, our findings need to be replicated. Generally, oxytocin has been suggested to exert its effects on HPA-axis (Bos et al., 2012) and ANS-functioning (Ditzen et al., 2013) by modulating amygdala activation thereby potentially enhancing stress-coping. Altered amygdala function has been reported in CD (Rogers and De Brito, 2016) and associated with oxytocin alterations in psychopathy (Dadds et al., 2014). Thus, the interaction effect of HPA-axis/ANS and oxytocin on CD risk in females may be related to their differential effects on amygdala activation. Furthermore, environmental risk such as trauma exposure has been suggested to attenuate oxytocin effects on cortisol (Fragkaki et al., 2018). Neuropeptide alterations may thus be related to early adversity exposure.

Hence, our final set of analyses explored whether the neuroendocrine measures we identified as associated with CD also mediated the effects of well-established early environmental risk factors on CD. Overall, no significant mediation effects were found. For stress system measures, this is in line with a recent meta-analysis reporting no correlation between early life stress and basal cortisol in population-based adult samples (Fogelman and Canli, 2018). However, we found effects of female sex hormones partly explaining the risk of related early environmental risk factors on CD in both sexes, which is surprisingly consistent with our observation of robust associations between female sex hormones and CD. In females, estradiol and progesterone partly explained the effects of exposure to early insufficient care and single parenthood on CD risk, respectively. In males, estradiol was found to partly explain the effects of parental psychopathology on risk for CD. Our findings of

altered female sex hormones in females and males with CD might thus be associated with the exposure to early adversity. Additionally, and only in females, oxytocin partly explained risk for CD for early insufficient care. The female-specific interaction effect of HPA-axis/ANS and oxytocin on CD risk may thus be associated with the experience of early adversity. Alterations in the oxytocin system have been recently related with exposure to early-life adversities and adolescent aggression (Fragkaki et al., 2018; Tobon et al., 2018). Overall, indication of possible mediating effects of neuroendocrinological measures were few and primarily observed in females. Therefore, no further mediation analyses with multiple mediators were performed. The underlying neurobiological mechanisms mediating environmental risk in CD might be less related to basal neuroendocrinological changes but more associated with neuroendocrine alterations in response to acute stress. Future research in females and males with CD investigating whether altered neuroendocrine responses to stress mediate the effects of especially family-related environmental risk factors is warranted.

This study had several strengths. We included a large and reliably diagnosed European sample of females and males with and without CD, the groups being matched for sex, age, and pubertal status, allowing sex-specific analyses. We assessed eight different established biomarkers of the HPA-axis, HPG-axis, and neuropeptide system, many of them for the first time in CD research (especially in females). In addition to testing single neuroendocrine measures as done previously, a major achievement of this study is the joint analysis of neuroendocrine biomarkers – deriving latent factors and testing for interactions between them. Importantly, we also controlled for key confounding variables on neuroendocrine functioning (e.g., smoking, BMI, assessment time). Finally, this is the first study exploring whether basal neuroendocrine markers mediate the effects of environmental risk factors for CD.

However, this study had several limitations: First, data were collected across various European sites contributing different sample sizes according to site-specific recruitment

possibilities. To ensure comparability and minimize variance across sites, standardized operating procedure manuals were followed during all assessments. External monitors ensured that each site adhered to the standardized procedures and entered data correctly. Site was also included as a covariate in the statistical analyses. Second, the overarching FemNAT-CD project aimed to address the lack of research in females with CD. Thus, this study included a larger sample of females compared to males, which might have influenced results and reduced statistical power to detect effects. The findings therefore need to be replicated in (ideally larger) sex-balanced samples. Third, the age range was rather large, but groups were matched for age, which was also included as a covariate in the statistical analyses. Furthermore, only participants with at least early-pubertal status were included to reduce variance. However, future studies should also consider the inclusion of pre-pubertal participants to fully investigate neuroendocrine developmental transitions. Fourth, obtaining neuroendocrine measures at just a single timepoint may have reduced reliability compared to multiple assessments. While most previous neuroendocrine studies in CD also applied single timepoint measures, future studies assessing basal hormones at several timepoints across the day are encouraged to replicate results and investigate dynamic interactions between neuroendocrine systems. Besides, assessment of stress hormone system measures may be more informative applying stress rather than basal assessment conditions. Fifth, methodological concerns of neuropeptide assessment have also been raised (MacLean et al., 2019; Martins et al., 2020) whereas other work supports salivary neuropeptide assessment as a reliable and valid method (Bernhard et al., 2018b; de Jong et al., 2015). While steroid hormones should be able to diffuse equally in all tissues, use of saliva for neuroendocrine assessments may not fully capture central release of neuropeptides (Jurek and Neumann, 2018). In a recent study positive correlations of salivary oxytocin with its central concentrations were only found for early morning levels (Kagerbauer et al., 2019), while in an earlier, but larger study modest to strong correlations were reported (Martin et al., 2018).

Recent findings of animal studies also support the notion that oxytocin may bypass the blood-brain-barrier (Lee et al., 2020). Overall, more work is needed to replicate current findings and extend knowledge about the reliability of particularly basal neuropeptide assessments. Sixth, saliva sample storage at -20°C for longer than six months was reported to be less optimal compared to -80°C storage (Granger et al., 2004). As sample storage did not differ between groups, possible degradation because of storage temperature may only have influenced absolute, rather than relative neuroendocrine levels. Seventh, pubertal status was assessed via self-report. Future studies using additional measures of pubertal development, such as physical examination by paediatricians, are encouraged to analyse its influence on basal neuroendocrinology in CD. Eighth, though our healthy control sample was not free of lifetime, e.g., internalizing mental disorders, the uneven distribution of comorbid psychiatric disorders between groups and sexes limited the inclusion of comorbid mental disorders as additional outcome variables in our statistical analyses. To fully investigate possible influences of psychiatric comorbidities, future studies including matched control subjects with/without CD and other common mental disorders are warranted. Finally, analyses were performed in an exploratory fashion, thus no correction for multiple comparisons was applied. Further studies (especially longitudinal as risk factors were assessed retrospectively) are strongly needed to replicate and extend current findings.

In conclusion, this study strongly enhances knowledge about the underlying neurobiology of CD. Our findings indicate sex-specific associations between basal neuroendocrine measures and CD in a large European sample. Especially androgen increases in males and estrogen reductions in females may contribute to CD-related behavioural symptoms. Only in females, antagonistic effects between the HPA-axis/ANS and oxytocin systems on CD risk were found, suggesting a possibly oxytocin dysregulation in females with CD. Results of neuroendocrine alterations are underscored by mediating effects of estradiol,

progesterone, and oxytocin for family-related environmental risk factors on CD. Our findings provide an important platform for future research investigating complex and sex-specific interactions between neuroendocrine systems in risk for CD with potentially wide-ranging implications for clinical practice and interventions.

References

American Psychiatric Association, 2000. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-TR). American Psychiatric Association, Washington, DC.

Angyal, N., Halasz, J., Meszaros, G., Kovacs, J.K., Kruk, E., Nemoda, Z., 2016. Potential salivary biomarkers and their genetic effects in a pilot study of adolescent boys with externalizing problems. Neuropsychopharmacol. Hungarica 18, 173–179.

Bakker-Huvenaars, M.J., Greven, C.U., Herpers, P., Baanders, A.N., Nijhof, K.S., Scheepers, F., Rommelse, N., Glennon, J.C., Buitelaar, J.K., 2020. Saliva oxytocin, cortisol, and testosterone levels in adolescent boys with autism spectrum disorder, oppositional defiant disorder/conduct disorder and typically developing individuals. Eur. Neuropsychopharmacol. 30, 87–101. doi:10.1016/j.euroneuro.2018.07.097

Bauer, A.M., Quas, J.A., Boyce, W.T., 2002. Associations between physiological reactivity and children's behavior: advantages of a multisystem approach. J. Dev. Behav. Pediatr. 23, 102–113. doi:10.1097/00004703-200204000-00007

Bernhard, A., Martinelli, A., Ackermann, K., Saure, D., Freitag, C.M., 2018a. Association of trauma, Posttraumatic Stress Disorder and Conduct Disorder: A systematic review and meta-analysis. Neurosci. Biobehav. Rev. 91, 153–169. doi:10.1016/j.neubiorev.2016.12.019

Bernhard, A., van der Merwe, C., Ackermann, K., Martinelli, A., Neumann, I.D., Freitag, C.M., 2018b. Adolescent oxytocin response to stress and its behavioral and endocrine correlates. Horm. Behav. 105, 157–165. doi:10.1016/j.yhbeh.2018.08.010

Bos, P.A., Panksepp, J., Bluthé, R.M., Honk, J. van, 2012. Acute effects of steroid hormones and neuropeptides on human social-emotional behavior: A review of single administration studies. Front. Neuroendocrinol. 33, 17–35. doi:10.1016/j.yfrne.2011.01.002

Bunea, I.M., Szentágotai-Tatar, A., Miu, A.C., 2017. Early-life adversity and cortisol response to social stress: a meta-analysis. Transl. Psychiatry 7, 1–8. doi:10.1038/s41398-017-0032-3

Burt, S.A., McGue, M., DeMarte, J.A., Krueger, R.F., Iacono, W.G., 2006. Timing of menarche and the origins of conduct disorder. Arch. Gen. Psychiatry 63, 890–896. doi:10.1001/archpsyc.63.8.890

Cappadocia, M.C., Desrocher, M., Pepler, D., Schroeder, J.H., 2009. Contextualizing the neurobiology of conduct disorder in an emotion dysregulation framework. Clin. Psychol. Rev. 29, 506–518. doi:10.1016/j.cpr.2009.06.001

Chistiakov, D.A., Chekhonin, V.P., 2019. Early-life adversity-induced long-term epigenetic programming associated with early onset of chronic physical aggression: Studies in humans and animals. World J Biol Psychiatry 20, 258–277. doi:10.1080/15622975.2017.1322714

Cohen, J., 1988. Statistical Power Analysis for the Behavioral Sciences, 2nd ed. Lawrence

Earlbaum Associates, Hillsdale.

Constantino, J.N., Grosz, D., Saenger, P., Chandler, D.W., Nandi, R., Earls, F.J., 1993. Testosterone and Aggression in Children. J. Am. Acadamic Child Adolesc. Psychiatry 32, 1217–1222.

Crider, A., Pillai, A., 2016. Estrogen Signaling as a Therapeutic Target in Neurodevelopmental Disorders. J. Pharmacol. Exp. Ther. 360, 48–58. doi:10.1124/jpet.116.237412

Cushing, B.S., Kramer, K.M., 2005. Mechanisms underlying epigenetic effects of early social experience: The role of neuropeptides and steroids. Neurosci. Biobehav. Rev. 29, 1089–1105. doi:10.1016/j.neubiorev.2005.04.001

Dadds, M.R., Moul, C., Cauchi, A., Dobson-stone, C., Hawes, D.J., Brennan, J., Urwin, R., Ebstein, R.E., 2014. Polymorphisms in the oxytocin receptor gene are associated with the development of psychopathy. Dev. Psychopathol. 26, 21–31. doi:10.1017/S0954579413000485

de Jong, T.R., Menon, R., Bludau, A., Grund, T., Biermeier, V., Klampfl, S.M., Jurek, B., Bosch, O.J., Hellhammer, J., Neumann, I.D., 2015. Salivary oxytocin concentrations in response to running, sexual self-stimulation, breastfeeding and the TSST: The Regensburg Oxytocin Challenge (ROC) study. Psychoneuroendocrinology 62, 381–388. doi:10.1016/j.psyneuen.2015.08.027

Dekkers, T.J., van Rentergem, J.A.A., Meijer, B., Popma, A., Wagemaker, E., Huizenga, H.M., 2019. A meta-analytical evaluation of the dual-hormone hypothesis: Does cortisol moderate the relationship between testosterone and status, dominance, risk taking, aggression, and psychopathy? Neurosci. Biobehav. Rev. 96, 250–271. doi:10.1016/j.neubiorev.2018.12.004

Ditzen, B., Nater, U.M., Schaer, M., Marca, L., Bodenmann, G., Ehlert, U., Heinrichs, M., 2013. Sex-specific effects of intranasal oxytocin on autonomic nervous system and emotional responses to couple conflict. Soc. Cogn. Affect. Neurosci. 8, 897–902. doi:10.1093/scan/nss083

Dmitrieva, T.N., Oades, R.D., Hauffa, B.P., Egers, C., 2001. Dehydroepiandrosterone sulphate (DHEA-S) and corticotropin levels are high in young male patients with conduct disorder: Comparisons for growth factors, thyroid and gonadal hormones. Neuropsychobiology 43, 134–140. doi:10.1159/000054881

Dorn, L.D., Kolko, D.J., Susman, E.J., Huang, B., Stein, H., Music, E., Bukstein, O.G., 2009. Salivary gonadal and adrenal hormone differences in boys and girls with and without disruptive behavior disorders: Contextual variants. Biol. Psychol. 81, 31–39. doi:10.1016/j.biopsycho.2009.01.004

Erskine, H.E., Ferrari, A.J., Polanczyk, G. V., Moffitt, T.E., Murray, C.J.L., Vos, T., Whiteford, H.A., Scott, J.G., 2014. The global burden of conduct disorder and attention-deficit/hyperactivity disorder in 2010. J. Child Psychol. Psychiatry Allied Discip. 55, 328–336. doi:10.1111/jcpp.12186

Fairchild, G., Baker, E., Eaton, S., 2018. Hypothalamic-Pituitary-Adrenal Axis Function in Children and Adults with Severe Antisocial Behavior and the Impact of Early Adversity. Curr. Psychiatry Rep. 20, 1–9.

Fairchild, G., Hawes, D.J., Frick, P.J., Copeland, W.E., Odgers, C.L., Franke, B., Freitag, C.M., De Brito, S.A., 2019. Conduct disorder. Nat. Rev. Dis. Prim. 5, 1–25. doi:10.1038/s41572-019-0095-y

Feilhauer, J., Cima, M., Korebrits, A., Nicolson, N.A., 2013. Salivary cortisol and psychopathy dimensions in detained antisocial adolescents. Psychoneuroendocrinology 38, 1586–1595. doi:10.1016/j.psyneuen.2013.01.005

Fogelman, N., Canli, T., 2018. Early life stress and cortisol: A meta-analysis. Horm. Behav. 98, 63–76. doi:10.1016/j.yhbeh.2017.12.014

Fragkaki, I., Cima, M., Granic, I., 2018. The role of trauma in the hormonal interplay of cortisol, testosterone, and oxytocin in adolescent aggression. Psychoneuroendocrinology 88, 24–37. doi:10.1016/j.psyneuen.2017.11.005

Freitag, C.M., Hänig, S., Schneider, A., Seitz, C., Palmason, H., Retz, W., Meyer, J., 2012. Biological and psychosocial environmental risk factors influence symptom severity and psychiatric comorbidity in children with ADHD. J. Neural Transm. 119, 81–94.

Freitag, C.M., Konrad, K., Stadler, C., De Brito, S.A., Popma, A., Herpertz, S.C., et Al., 2018. Conduct disorder in adolescent females-current state of research and study design of the Fem-NAT-CD consortium. Eur. Child Adolesc. Psychiatry 27, 1077–1093.

Granger, D.A., Shirtcliff, E.A., Booth, A., Kivlighan, K.T., Schwartz, E.B., 2004. The "trouble" with salivary testosterone. Psychoneuroendocrinology 29, 1229–1240. doi:10.1016/j.psyneuen.2004.02.005

Gunnar, M.R., Quevedo, K., 2007. The neurobiology of stress and development. Annu. Rev. Psychol. 58, 145–173. doi:10.1146/annurev.psych.58.110405.085605

Jurek, B., Neumann, I.D., 2018. The oxytocin receptor: From intracellular signaling to behavior. Physiol. Rev. 98, 1805–1908. doi:10.1152/physrev.00031.2017

Kagerbauer, S.M., Debus, J.M., Martin, J., Gempt, J., Jungwirth, B., Hapfelmeier, A., Podtschaske, A.H., 2019. Absence of a diurnal rhythm of oxytocin and arginine-vasopressin in human cerebrospinal fluid, blood and saliva. Neuropeptides 78, 101977. doi:10.1016/j.npep.2019.101977

Kohls, G., Baumann, S., Gundlach, M., Scharke, W., Bernhard, A., Martinelli, A., Ackermann, K., Kersten, L., Prätzlich, M., Oldenhof, H., Jansen, L., van den Boogaard, L., Smaragdi, A., Gonzalez-Madruga, K., Cornwell, H., Rogers, J.C., Pauli, R., Clanton, R., Baker, R., Bigorra, A., Kerexeta-Lizeaga, I., Sesma-Pardo, E., Aguirregomoscorta-Menéndez, F., Siklósi, R., Dochnal, R., Kalogerakis, Z., Pirlympou, M., Papadakos, L., Dikeos, D., Hervas, A., Herpertz-Dahlmann, B., Fernández-Rivas, A., Popma, A., Stadler, C., De Brito, S.A., Blair, J.R., Freitag, C.M., Fairchild, G., Konrad, K., 2020. Investigating Sex Differences in Emotion Recognition, Learning, and Regulation Among Youths With Conduct Disorder. J. Am. Acad. Child Adolesc. Psychiatry 59, 263–273. doi:10.1016/j.jaac.2019.04.003

Koss, K.J., Gunnar, M.R., 2018. Early adversity, the hypothalamic–pituitary–adrenocortical axis, and child psychopathology. J Child Psychol Psychiatr 59, 327–346. doi:10.1111/jcpp.12784

Kudielka, B.M., Gierens, A., Hellhammer, D.H., Wüst, S., Schlotz, W., 2012. Salivary

cortisol in ambulatory assessment-some dos, some don’ts, and some open questions. Psychosom. Med. 74, 418–431. doi:10.1097/PSY.0b013e31825434c7

Lee, M.R., Shnitko, T.A., Blue, S.W., Kaucher, A. V., Winchell, A.J., Erikson, D.W., Grant, K.A., Leggio, L., 2020. Labeled oxytocin administered via the intranasal route reaches the brain in rhesus macaques. Nat. Commun. 11, 1–10. doi:10.1038/s41467-020-15942-1

Levy, T., Bloch, Y., Bar-Maisels, M., Gat-Yablonski, G., Djalovski, A., Borodkin, K., Apter, A., 2015. Salivary oxytocin in adolescents with conduct problems and callous-unemotional traits. Eur. Child Adolesc. Psychiatry 24, 1543–1551. doi:10.1007/s00787-015-0765-6

MacLean, E.L., Wilson, S.R., Martin, W.L., Davis, J.M., Nazarloo, H.P., Carter, C.S., 2019. Challenges for measuring oxytocin: The blind men and the elephant? Psychoneuroendocrinology 107, 225–231. doi:10.1016/j.psyneuen.2019.05.018

Martin, J., Kagerbauer, S.M., Gempt, J., Podtschaske, A., Hapfelmeier, A., Schneider, G., 2018. Oxytocin levels in saliva correlate better than plasma levels with concentrations in the cerebrospinal fluid of patients in neurocritical care. J. Neuroendocrinol. 30, e12596. doi:10.1111/jne.12596

Martins, D., Gabay, A.S., Mehta, M., Paloyelis, Y., 2020. Salivary and plasmatic oxytocin are not reliable trait markers of the physiology of the oxytocin system in humans. Elife 9, 1–19. doi:10.7554/ELIFE.62456

Maughan, B., Rowe, R., Messer, J., Goodman, R., Meltzer, H., 2004. Conduct Disorder and Oppositional Defiant Disorder in a national sample: developmental epidemology. J. Child Psychol. Psychiatry 45, 609–621.

Montoya, E.R., Bos, P.A., 2017. How Oral Contraceptives Impact Social-Emotional Behavior and Brain Function. Trends Cogn. Sci. 21, 125–136. doi:10.1016/j.tics.2016.11.005

Nater, U.M., Rohleder, N., 2009. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. Psychoneuroendocrinology 34, 486–496. doi:10.1016/j.psyneuen.2009.01.014

Nguyen, T.V., McCracken, J.T., Albaugh, M.D., Botteron, K.N., Hudziak, J.J., Ducharme, S., 2016. A testosterone-related structural brain phenotype predicts aggressive behavior from childhood to adulthood. Psychoneuroendocrinology 63, 109–118. doi:10.1016/j.psyneuen.2015.09.021

Northover, C., Thapar, A., Langley, K., Fairchild, G., van Goozen, S.H.M., 2016. Cortisol levels at baseline and under stress in adolescent males with attention-deficit hyperactivity disorder, with or without comorbid conduct disorder. Psychiatry Res. 242, 130–136. doi:10.1016/j.psychres.2016.05.052

Oldenhof, H., Prätzlich, M., Ackermann, K., Baker, R., Batchelor, M., Baumann, S., Bernhard, A., Clanton, R., Dikeos, D., Dochnal, R., Fehlbaum, L.V., Fernández-Rivas, A., de Geus, E., Gonzalez, K., de Artaza-Lavesa, M.G., Guijarro, S., Gundlach, M., Herpertz-Dahlmann, B., Hervas, A., Jansen, L., Kersten, L., Kohls, G., Konsta, A., Lazaratou, H., Kerexeta-Lizeaga, I., Martinelli, A., van Nimwegen, T., Puzzo, I., Raschle, N.M., Rogers, J., Siklósi, R., Smaragdi, A., Steppan, M., De Brito, S., Fairchild, G., Kieser, M., Konrad, K., Freitag, C., Stadler, C., Popma, A., 2019. Baseline autonomic nervous system activity in female children and adolescents with conduct

disorder: Psychophysiological findings from the FemNAT-CD study. J. Crim. Justice 101564. doi:10.1016/j.jcrimjus.2018.05.011

Oyola, M.G., Handa, R.J., 2017. Hypothalamic–pituitary–adrenal and hypothalamic–pituitary– gonadal axes: sex differences in regulation of stress responsivity. Stress 20, 476–494. doi:10.1080/10253890.2017.1369523.Hypothalamic

Pajer, K., Tabbah, R., Gardner, W., Rubin, R.T., Kenneth Czambel, R., Wang, Y., 2006. Adrenal androgen and gonadal hormone levels in adolescent girls with conduct disorder. Psychoneuroendocrinology 31, 1245–1256. doi:10.1016/j.psyneuen.2006.09.005

Petersen, A.C., Crockett, L., Richards, M., Boxer, A., 1988. A Self-Report Measure of Pubertal Status: Reliability, Validity, and Initial Norms. J. Youth Adolesc. 17, 117–133.

Prätzlich, M., Oldenhof, H., Steppan, M., Ackermann, K., Baker, R., Batchelor, M., Baumann, S., Bernhard, A., Clanton, R., Dikeos, D., Dochnal, R., Fehlbaum, L.V., Fernández-Rivas, A., González de Artaza-Lavesa, M., Gonzalez-Madruga, K., Guijarro, S., Gundlach, M., Herpertz-Dahlmann, B., Hervas, A., Jansen, L., Kerexeta-Lizeaga, I., Kersten, L., Kirchner, M., Kohls, G., Konsta, A., Lazaratou, H., Martinelli, A., Menks, W.M., Puzzo, I., Raschle, N.M., Rogers, J., Siklósi, R., Smaragdi, A., Vriends, N., Konrad, K., De Brito, S., Fairchild, G., Kieser, M., Freitag, C.M., Popma, A., Stadler, C., 2019. Resting autonomic nervous system activity is unrelated to antisocial behaviour dimensions in adolescents: Cross-sectional findings from a European multi-centre study. J. Crim. Justice 65, 101536. doi:10.1016/j.jcrimjus.2018.01.004

Preacher, K.J., Hayes, A.F., 2008. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. Behav. Res. Methods 40, 879–891. doi:10.3758/BRM.40.3.879

Rivenbark, J.G., Odgers, C.L., Caspi, A., Harrington, H.L., Hogan, S., Houts, R.M., Poulton, R., Moffitt, T.E., 2018. The high societal costs of childhood conduct problems: evidence from administrative records up to age 38 in a longitudinal birth cohort. J. Child Psychol. Psychiatry Allied Discip. 59, 703–710. doi:10.1111/jcpp.12850

Rogers, J.C., De Brito, S.A., 2016. Cortical and Subcortical Gray Matter Volume in Youths with Conduct Problems A Meta-analysis. JAMA Psychiatry 73, 64–72. doi:10.1001/jamapsychiatry.2015.2423

Salimetrics, 2021. Calculating inter-and intra-assay-coefficients of variability. https://salimetrics.com/calculating-inter-and-intra-assay-coefficients-of-variability/.

Soma, K.K., Rendon, N.M., Boonstra, R., Albers, H.E., Demas, G.E., 2015. DHEA effects on brain and behavior: Insights from comparative studies of aggression. J. Steroid Biochem. Mol. Biol. 145, 261–272.

Spiteri, T., Musatov, S., Ogawa, S., Ribeiro, A., Pfaff, D.W., Ågmo, A., 2010. The role of the estrogen receptor α in the medial amygdala and ventromedial nucleus of the hypothalamus in social recognition, anxiety and aggression. Behav. Brain Res. 210, 211–220. doi:10.1016/j.bbr.2010.02.033

Tobon, A.L., Newport, D.J., Nemeroff, C.B., 2018. The Role of Oxytocin in Early Life Adversity and Later Psychopathology: a Review of Preclinical and Clinical Studies. Curr Treat Options Psych 5, 401–415. doi:10.1007/s40501-018-0158-9

Yildirim, B.O., Derksen, J.J.L., 2012. A review on the relationship between testosterone and the interpersonal/affective facet of psychopathy. Psychiatry Res. 197, 181–198. doi:10.1016/j.psychres.2011.08.016

Young, R., Sweeting, H., West, P., 2012. Associations between DSM-IV diagnosis, psychiatric symptoms and morning cortisol levels in a community sample of adolescents. Soc. Psychiatry Psychiatr. Epidemiol. 47, 723–733. doi:10.1007/s00127-011-0374-8

Table 1. Descriptive statistics for demographic and clinical characteristics, environmental risk factors, and psychiatric comorbidities in females and males with Conduct Disorder (CD) compared to healthy controls (HCs).

	Females (N=216)			**Males (N=144)**			**All (N=360)**		
	CD (N=99)	HCs (N=117)	*F*/*X*²	CD (N=67)	HCs (N=77)	*F*/*X*²	CD (N=166)	HCs (N=194)	*F*/*X*²
Sample characteristics									
Age (years), mean (SD), y	15.24 (2.1)	15.71 (1.9)	2.96	14.87 (2.4)	14.84 (2.1)	<0.01	15.09 (2.2)	15.37 (2.0)	1.53
Estimated Full-scale IQ, mean (SD)	93.57 (13.2)	104.39 (12.7)	37.50***	98.75 (14.5)	105.32 (11.8)	9.02**	95.66 (14.0)	104.76 (12.3)	43.25***
Body Mass Index, mean (SD)	22.61 (4.9)	21.93 (3.9)	1.25	20.16 (2.9)	20.70 (3.3)	1.01	21.64 (4.4)	21.46 (3.7)	0.17
Pubertal Status, N (%)			0.33			0.63			0.30
Early-pubertal	1 (1.0)	2 (1.7)		16 (23.9)	19 (24.7)		17 (10.2)	21 (10.8)	
Mid-pubertal	6 (6.1)	6 (5.1)		19 (28.4)	23 (29.9)		25 (15.1)	29 (14.9)	
Late-pubertal	68 (68.7)	79 (67.5)		30 (44.8)	31 (40.3)		98 (59.0)	110 (56.7)	
Post-pubertal	24 (24.2)	30 (25.6)		2 (3.0)	4 (5.2)		26 (15.7)	34 (17.5)	
Current smoking, N (%)	61 (62.2)	14 (12.1)	58.75***	37 (56.9)	8 (10.4)	35.26***	98 (60.1)	22 (11.4)	93.88***
Any medication, N (%)	33 (33.3)	7 (6.0)	26.59***	24 (35.8)	2 (2.6)	26.73***	57 (34.3)	9 (4.6)	52.70***
Risk factors									
Prenatal smoking, N (%)	31 (38.3)	15 (13.6)	15.49***	28 (50.9)	9 (12.2)	23.16***	59 (43.4)	24 (13.0)	37.47***
Prenatal violence, N (%)	23 (28.8)	8 (7.2)	15.87***	11 (20.8)	4 (5.4)	6.99**	34 (25.6)	12 (6.5)	22.76***
Maternal age at birth, mean (SD)	26.48 (6.2)	30.64 (5.3)	25.10***	28.06 (5.5)	31.42 (5.2)	12.37**	27.10 (6.0)	30.95 (5.3)	37.53***
Single parenthood 1st year, N (%)	22 (25.9)	10 (8.9)	10.21**	18 (34.6)	4 (5.4)	18.08***	40 (29.2)	14 (7.5)	26.61***
Early insufficient care 1st year, N (%)	10 (11.9)	6 (5.5)	2.56	8 (15.1)	1 (1.4)	8.72**	18 (13.1)	7 (3.8)	9.35**
Adverse family situation, N (%)			29.37***			7.96***			55.69***
Either disharmony or isolation	21 (26.6)	20 (18.7)		20 (41.7)	17 (23.6)		41 (32.3)	37 (20.7)	
Both disharmony and isolation	23 (29.1)	4 (3.7)		15 (31.3)	3 (4.2)		38 (29.9)	7 (3.9)	
Parental education, mean (SD)	2.92 (1.1)	3.92 (1.0)	44.54***	3.08 (0.8)	4.00 (1.1)	28.90***	2.99 (1.0)	3.95 (1.0)	73.69***
Parental psychopathology[a], N (%)			9.36*			8.61*			17.77***
1 out of 3 categories	10 (14.9)	7 (6.4)		8 (16.7)	5 (6.9)		18 (15.7)	12 (6.6)	
2 out of 3 categories	7 (10.4)	3 (2.7)		5 (10.4)	1 (1.4)		12 (10.4)	4 (2.2)	
3 out of 3 categories	2 (3.0)	2 (1.8)		1 (2.1)	1 (1.4)		3 (2.6)	3 (1.6)	
Number traumatic events, mean (SD)	2.59 (2.1)	0.79 (1.0)	69.57***	2.57 (1.9)	1.18 (1.1)	30.14***	2.58 (2.0)	0.95 (1.0)	98.93***
Lifetime ADHD, N (%)	39 (39.4)	0 (0.0)	56.25***	38 (56.7)	0 (0.0)	59.33***	77 (46.4)	0 (0.0)	114.47***
Lifetime SUD, N (%)	20 (20.2)	0 (0.0)	26.05***	21 (31.3)	1 (1.3)	24.99***	41 (24.7)	1 (0.5)	50.77***
Lifetime anxiety disorder[b], N (%)	16 (16.2)	4 (3.4)	10.36**	15 (22.4)	3 (3.9)	11.20**	31 (18.7)	7 (3.6)	21.51***
Lifetime depressive disorder, N (%)	38 (38.4)	1 (0.9)	51.05***	15 (22.4)	0 (0.0)	19.24***	53 (31.9)	1 (0.5)	69.23***
Lifetime PTSD, N (%)	16 (16.2)	0 (0.0)	20.42***	7 (10.4)	0 (0.0)	8.46**	23 (13.9)	0 (0.0)	28.71***

Note. ADHD=Attention Deficit Hyperactivity Disorder, PTSD=Posttraumatic Stress Disorder, SD=standard deviation, SUD=Substance Use Disorder (including substance use and dependence). ***p<.001, **p <.01, *p<.05. [a]For description of categories, please see Supplement 2. [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 2. Multivariable logistic regression analyses of neuroendocrine measures as predictors of Conduct Disorder (CD).

Neuroendocrine measures	**Females**				**Males**			
	Odds ratio	95%CI LB	95%CI UB	*p*	Odds ratio	95%CI LB	95%CI UB	*p*
zlogCortisol	1.11	0.69	1.77	.67	0.98	0.51	1.87	.95
zlogAlpha-Amylase	1.03	0.70	1.52	.87	0.60	0.34	1.07	.08
zlogTestosterone	1.69	0.77	3.71	.19	1.63	0.71	3.73	.25
zlogDHEA-S	1.18	0.75	1.87	.47	1.76	0.94	3.29	.08
zlogEstradiol	0.57	0.34	0.97	.04*	0.60	0.28	1.28	.19
zlogProgesterone	0.84	0.50	1.39	.49	0.89	0.35	2.26	.80
zlogArginine-Vasopressin	0.97	0.62	1.51	.88	1.00	0.64	1.56	>.99
zlogOxytocin	1.04	0.70	1.56	.83	0.86	0.52	1.42	.56

Note. CI=confidence interval, DHEA-S=dehydroepiandrosterone-sulfate, LB=lower bound, UB=upper bound, zlog=z-standardized and log-transformed. *p<.05.

Table 3. Rotated factor pattern of neuroendocrine measures in females and males.

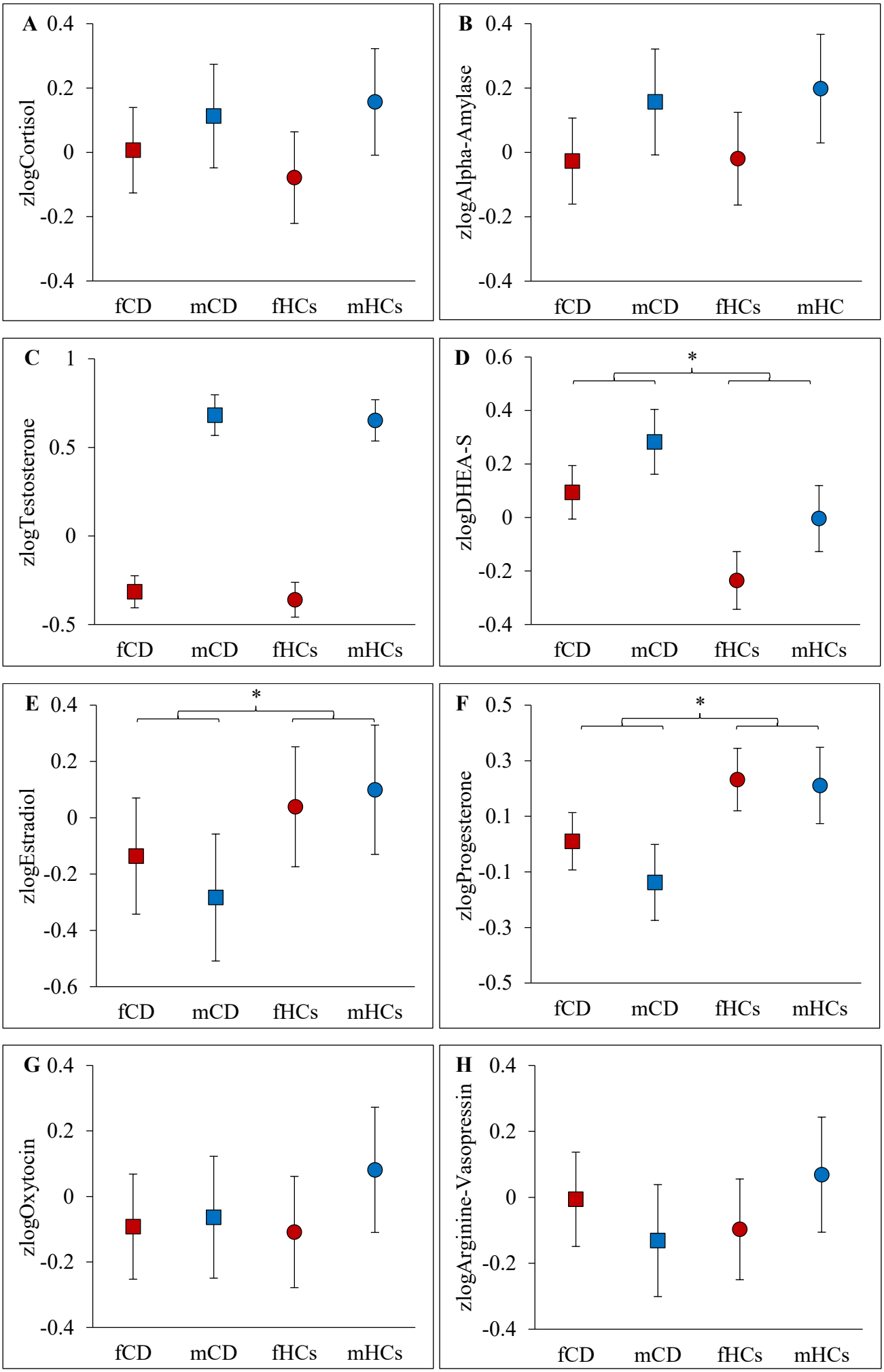
Neuroendocrine measures	**Females**				**Males**			
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 1	Factor 2	Factor 3	Factor 4
zlogCortisol	0.47	0.08	**0.62**	0.14	0.46	0.50	-0.36	-0.30
zlogAlpha-Amylase	-0.13	-0.02	**0.87**	-0.11	-0.01	0.01	**0.81**	0.09
zlogTestosterone	**0.80**	0.24	0.14	0.13	0.33	**0.82**	0.09	0.01
zlogDHEA-S	0.24	**0.71**	0.16	0.31	-0.11	**0.87**	0.16	0.11
zlogEstradiol	**0.79**	0.09	-0.13	-0.11	**0.88**	<0.01	0.20	0.05
zlogProgesterone	**0.84**	-0.16	0.10	-0.14	**0.88**	0.18	-0.11	-0.09
zlogArginine-Vasopressin	-0.07	**0.80**	-0.10	-0.25	-0.07	-0.16	**-0.58**	0.21
zlogOxytocin	-0.11	-0.03	-0.04	**0.90**	-0.03	0.08	-0.08	**0.94**

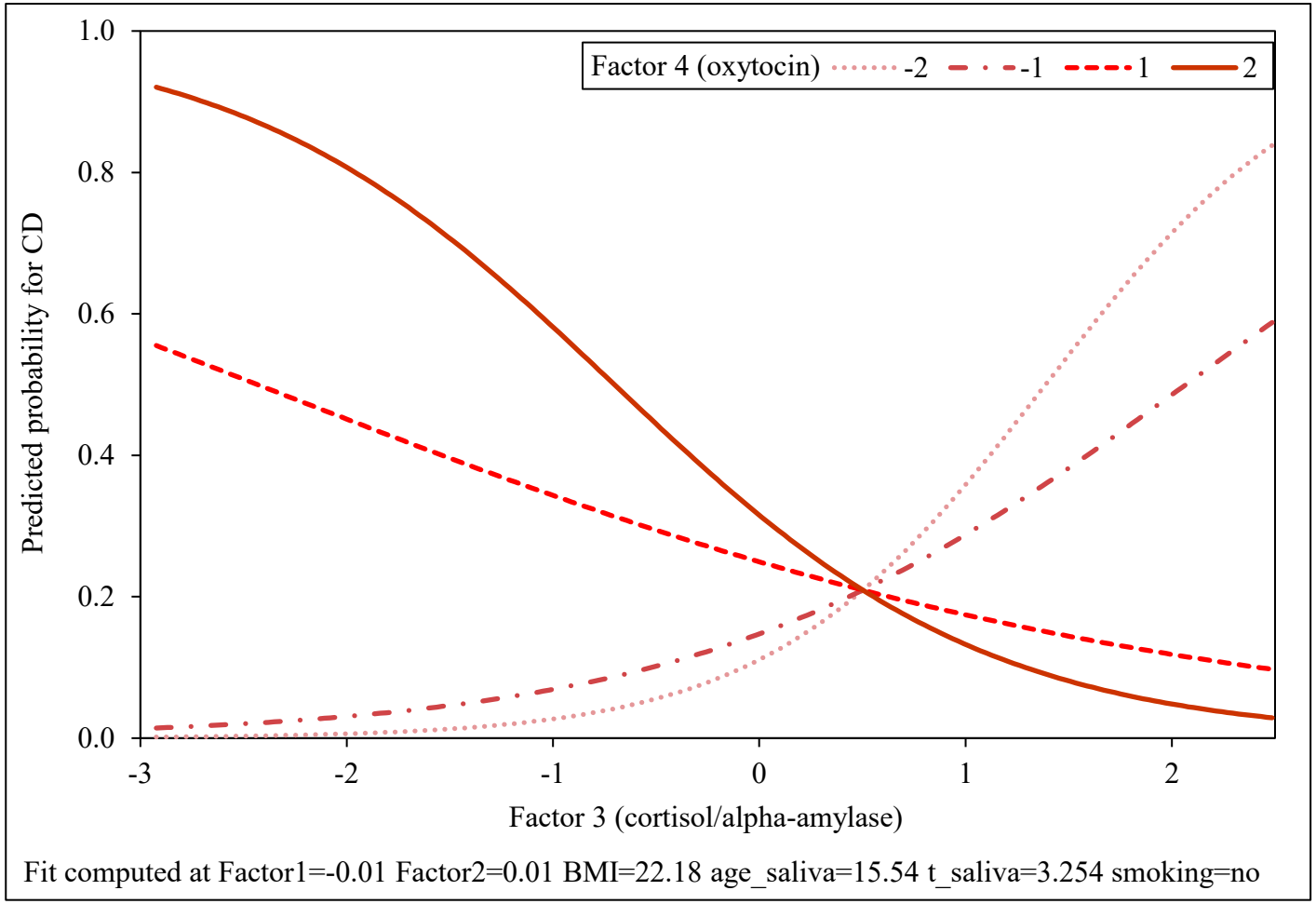
Note. DHEA-S=dehydroepiandrosterone-sulfate, zlog=z-standardized and log-transformed.

Figure titles and legends

Fig. 1 Baseline hormone and neuropeptide levels in Conduct Disorder (CD) participants compared to healthy controls (HCs). Salivary levels of cortisol (A), alpha-amylase (B), testosterone (C), dehydroepiandrosterone-sulfate (DHEA-S; D), estradiol (E), progesterone (F), oxytocin (G), and arginine-vasopressin (H). Data are shown as least square means of univariate analyses of covariance (ANCOVA), with z-standardized and log-transformed (zlog) neuroendocrine levels ± Standard Error of Means, for easier interpretation of results according to statistical analyses. Original, untransformed data is presented in Supplementary Table S2. *=Significant effect of group for the respective ANCOVA with group (CD vs. HCs) and sex (female vs. male) as between-subject factors, controlled for major confounder (see Statistical Analyses). $p<.05$. fCD=female CD, mCD=male CD, fHCs=female HCs, mHCs=male HCs.

Fig. 2 Interaction between cortisol/alpha-amylase and oxytocin as a predictor of Conduct Disorder (CD) in females. Significant interaction effect of multivariable logistic regression analysis after principal component analysis of Factor 3 (cortisol/alpha-amylase) and Factor 4 (oxytocin) for risk of CD in females. Risk for CD was lowest in females with parallel cortisol/alpha-amylase and oxytocin activation (either low or high; e.g. negative scores along the x-axis for Factor 3, and solid and dotted lines -2 and -1 for Factor 4), and highest in individuals with either high cortisol/alpha-amylase and low oxytocin, or vice versa, suggesting contrasting activation of these systems (i.e., negative scores along the x-axis for Factor 3, and dashed lines 1 and 2 for Factor 4). Age_saliva=age at saliva sampling, BMI=body mass index, smoking=current smoking, t_saliva=time of saliva sampling.


A
zlogCortisol
0.4
0.2
0
-0.2
-0.4
fCD
mCD
fHCs
mHCs
B
zlogAlpha-Amylase
0.4
0.2
0
-0.2
-0.4
fCD
mCD
fHCs
mHC
C
zlogTestosterone
1
0.5
0
-0.5
fCD
mCD
fHCs
mHCs
D
zlogDHEA-S
*
0.6
0.4
0.2
0
-0.2
-0.4
fCD
mCD
fHCs
mHCs
E
zlogEstradiol
*
0.4
0.2
0
-0.2
-0.4
-0.6
fCD
mCD
fHCs
mHCs
F
zlogProgesterone
*
0.5
0.3
0.1
-0.1
-0.3
-0.5
fCD
mCD
fHCs
mHCs
G
zlogOxytocin
0.4
0.2
0
-0.2
-0.4
fCD
mCD
fHCs
mHCs
H
zlogArginine-Vasopressin
0.4
0.2
0
-0.2
-0.4
fCD
mCD
fHCs
mHCs



Fit computed at Factor1=-0.01 Factor2=0.01 BMI=22.18 age_saliva=15.54 t_saliva=3.254 smoking=no

Author Disclosures

Role of funding source

This study was funded by the European Commission's Seventh Framework Program (FP7 grant no. 602407; FemNAT-CD). The European Commission had no further role in study design; in the collection, analysis and interpretation of the data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

Authors AB and CMF designed the study. All authors were involved in the data collection. Authors AB, CMF and MK planned and undertook the statistical analysis. Author AB made the graphical representation of the data and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of Interest

Author SdB has received speaker fees from the Child Mental Health Centre and the Centre for Integrated Molecular Brain Imaging. Author CMF receives royalties for books on Autism Spectrum Disorder, ADHD, and depressive disorder. Author SH receives royalties for books on eating disorders and textbooks on child and adolescent psychiatry, and speaker fees from Ferring Pharmaceuticals. Author AH has received travel grants from Takeda and consultancy from Exeltis. Author KK has received speaker fees from Shire Pharmaceuticals and Medice and receives royalties for books. Author CS receives royalties for a book on aggression. All other authors reported no biomedical financial interests or potential conflict of interests.

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Supplemental material for

"Sex-specific associations of basal steroid hormones and neuropeptides with Conduct Disorder and neuroendocrine mediation of environmental risk"

Additional information about the assessment of psychiatric disorders and IQ

Current and lifetime psychiatric disorders were assessed with the Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime version (K-SADS-PL[1]), a semi-structured diagnostic interview conducted separately with the adolescent and parents/caregivers by trained staff. Inter-rater reliability (Cohen's κ=.91) and agreement (95%) were high for CD (N=75). For comorbid psychiatric disorders (e.g., ADHD, oppositional defiant disorder, major depressive 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-Fourth Edition (11-16 years[2]; >16 years[3]). English sites used the Wechsler Abbreviated Scale of Intelligence[4].

Additional information about the assessment of environmental risk factors

Environmental risk factors were measured using a structured medical history interview[5] conducted by trained staff, designed to assess current and past psychosocial and medical risk factors of children and adolescents. Parents or caregivers were interviewed regarding: 1. pregnancy, 2. birth history and first year of life, 3. developmental milestones, 4. nursery and kindergarten, 5. school career, 6. chronic medical problems, 7. social information (incl. parental educational status), and 8. the presence of psychiatric disorders in the family. Based on thorough analyses of previous studies (e.g.[6]), a set of pre- and postnatal risk factors especially relevant for

conduct disorder were selected. These included risk factors in pregnancy and early development as well as family situation related risk factors. All items that showed a differential distribution between conduct disorder and controls were included in the data analyses. The environmental risk factors prenatal smoking and violence exposure during pregnancy, maternal age at birth, single parenthood (single mother or father) and early insufficient care (frequent change of caregivers) during participants' first year of life, were included as binary items (yes, no). The risk factors adverse family situation (familial disharmony and isolation, range=0-2) and parental psychopathology (maternal psychiatric disorder during and after the first year of the child's life, paternal psychiatric disorder in the first year after the child's birth; range=0-3) include summary scores with three and respectively four categories (0=no yes responses, 1=yes to one question, 2=yes to two questions, 3=yes to three questions) as the respective binary items correlated higher than $r>0.3$. 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[7]. 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[7]. Traumatic events from the Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime version (K-SADS-PL[1]) diagnostic interview for DSM-IV-TR are based on a list of 11 possible traumatic experiences: car accident, other accident, fire, witness of a disaster, witness of a violent crime, victim of a violent crime, confronted with traumatic news, witness to domestic violence, physical abuse, sexual abuse, other).

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

Site/*N*	Females		Males			
	CD	HCs	CD	HCs	∑	
Frankfurt am Main, Germany	17	39	23	29	108	X^2=34.27 (p<.001)
Aachen, Germany	21	20	23	26	90	
Amsterdam, The Netherlands	15	3	2	0	20	
Southampton, United Kingdom	9	13	12	19	53	
Basel, Switzerland	6	19	0	0	25	
Birmingham, United Kingdom	1	3	0	0	4	
Barcelona, Spain	4	1	1	0	6	
Bilbao, Spain	15	5	6	3	29	
Szeged, Hungary	5	5	0	0	10	
Athens, Greece	6	9	0	0	15	
∑	99	117	67	77	360	

Note. CD=conduct disorder, HCs=healthy controls.

Table S2. Untransformed data for each of the neuroendocrine measures in females and males with Conduct Disorder (CD) compared to healthy controls (HCs).

Neuroendocrine measures	Females (N=216)			Males (N=144)			All (N=360)		
	CD (N=99)	HCs (N=117)		CD (N=67)	HCs (N=77)		CD (N=166)	HCs (N=194)	
	Mean (SD)	*Mean (SD)*	*p*	*Mean (SD)*	*Mean (SD)*	*p*	*Mean (SD)*	*Mean (SD)*	*p*
Cortisol[a]	3.09 (2.02)	2.86 (2.83)	.05	3.11 (2.39)	2.98 (2.63)	.39	3.10 (2.17)	2.91 (2.75)	.04
Alpha-Amylase[b]	83.90 (70.39)	72.77 (69.84)	.08	90.58 (68.19)	83.37 (57.32)	.87	86.59 (69.38)	76.98 (65.21)	.15
Testosterone[c]	51.97 (23.53)	45.33 (15.41)	.10	96.20 (59.80)	89.82 (70.62)	.31	69.82 (47.25)	62.99 (50.82)	.12
DHEA-S[c]	5722.27 (4232.96)	4539.92 (3441.77)	.04	6161.26 (4376.30)	5301.34 (4282.41)	.15	5899.45 (4283.65)	4842.14 (3805.37)	.01
Estradiol[c]	2.47 (1.76)	2.63 (1.76)	.41	2.00 (1.30)	2.67 (3.64)	.10	2.28 (1.60)	2.65 (2.66)	.10
Progesterone[c]	108.82 (96.40)	115.18 (121.21)	.42	72.18 (58.34)	94.64 (66.35)	.02	94.03 (84.89)	107.03 (103.28)	.03
Oxytocin[c]	1.07 (0.38)	1.01 (0.39)	.13	1.12 (0.41)	1.19 (0.47)	.64	1.09 (0.39)	1.08 (0.43)	.35
Arginine-Vasopressin[c]	2.94 (1.41)	3.22 (1.69)	.35	2.95 (1.76)	3.41 (2.04)	.15	2.94 (1.55)	3.30 (1.83)	.10

Note. Statistical values are based on non-parametric Mann-Whitney-U-tests due to a non-normal distribution. DHEA-S=dehydroepiandrosterone-sulfate. [a]=in nmol/l, [b]=in U/ml, [c]=in pg/ml.

Table S3. Medication use by group and sex.

	Females			Males			All		
N (%)	CD	HCs	X^2	CD	HCs	X^2	CD	HCs	X^2
No medication	66 (66.7)	110 (94.0)	26.59***	43 (64.2)	75 (97.4)	26.73***	109 (65.7)	185 (95.4)	52.70***
Neuroleptics	12 (12.1)	0 (0.0)	15.02***	7 (10.4)	0 (0.0)	8.46**	19 (11.4)	0 (0.0)	23.44***
Stimulants	11 (11.1)	0 (0.0)	13.70**	16 (23.9)	0 (0.0)	20.69***	27 (16.3)	0 (0.0)	34.11***
SSRIs/antidepressants	10 (10.1)	0 (0.0)	12.39***	5 (7.5)	0 (0.0)	5.95*	15 (9.0)	0 (0.0)	18.29***
Other	5 (5.1)	7 (6.0)	0.09	2 (3.0)	2 (2.6)	0.02	7 (4.2)	9 (4.6)	0.38

Note. CD=Conduct Disorder, HCs=healthy controls. "Other" includes medication such as asthma medication, painkiller, or vitamin preparation. Participants may have been taking more than one medication category, thus the percentages do not sum to 100%. *p<.05, **p <.01, ***p<.001.

Table S4. Univariate analyses of covariance of neuroendocrine measures in females and males with Conduct Disorder (CD) compared to healthy controls (HCs).

Neuroendocrine measures	CD		HCs									
	Females	Males	Females	Males	Group			Sex			Group x sex	
	Least square means [LB 95%CI;UB 95%CI]				*F*	*p*	*d*	*F*	*p*	*d*	*F*	*p*
zlogCortisol	0.01 [-0.28;0.30]	0.11 [-0.22;0.45]	-0.08 [-0.38;0.23]	0.16 [-0.19;0.50]	0.03	.87	0.02	2.23	.14	-0.17	0.39	.53
zlogAlpha-Amylase	-0.03 [-0.30;0.25]	0.16 [-0.18;0.49]	-0.02 [-0.32;0.28]	0.20 [-0.15;0.54]	0.03	.86	-0.02	2.63	.11	-0.18	0.02	.88
zlogTestosterone	-0.32 [-0.50;-0.13]	0.68 [0.45;0.91]	-0.36 [-0.56;-0.16]	0.65 [0.42;0.89]	0.12	.73	0.04	111.91	<.001	-1.18	0.01	.93
zlogDHEA-S	0.09 [-0.10;0.29]	0.28 [0.05;0.52]	-0.24 [-0.45;-0.02]	0.00 [-0.25;0.24]	5.91	.02	0.27	3.78	.05	-0.22	0.04	.84
zlogEstradiol	-0.14 [-0.59;0.32]	-0.28 [-0.76;0.20]	0.04 [-0.42;0.50]	0.10 [-0.39;0.59]	4.97	.03	-0.24	0.15	.70	0.04	1.02	.31
zlogProgesterone	0.01 [-0.20;0.22]	-0.14 [-0.42;0.15]	0.23 [0.00;0.46]	0.21 [-0.08;0.50]	5.28	.02	-0.25	0.57	.45	0.08	0.38	.54
zlogOxytocin	-0.09 [-0.44;0.25]	-0.06 [-0.45;0.32]	-0.11 [-0.47;0.25]	0.08 [-0.31;0.48]	0.24	.62	-0.05	0.85	.36	-0.10	0.56	.45
zlogArginine-Vasopressin	-0.01 [-0.30;0.29]	-0.13 [-0.47;0.21]	-0.10 [-0.41;0.22]	0.07 [-0.28;0.42]	0.18	.67	-0.05	0.03	.86	-0.02	1.86	.17

Note. Results of univariate analyses of covariance with group (CD vs. HCs) and sex (female vs. male) as between-subject factors, and current smoking, body mass index, age, time of saliva sampling, and assessment site as covariates. CI=confidence interval, *d*=Cohen's d, DHEA-S=dehydroepiandrosterone-sulfate, LB=lower bound, UB=upper bound, zlog=z-standardized and log-transformed.

Table S5. Neuroendocrine measures as mediators of environmental risk on Conduct Disorder in females and males.

	Indirect effect	*95% CI [LB;UB]*	Direct effect	*p*	Total effect	*p*	Proportion of total effect mediated [%]
FEMALES							
zlogCortisol							
Prenatal smoking	0.00	[-0.02;0.02]	0.16	.05	0.16	.05	0.52
Prenatal violence	0.00	[-0.02;0.02]	0.20	.01	0.20	.01	0.97
Maternal age at birth	0.00	[-0.01;0.03]	-0.27	<.001	-0.27	<.001	0.46
Single parenthood	0.00	[-0.02;0.01]	0.14	.08	0.13	.08	1.57
Early insufficient care	0.00	[-0.02;0.01]	0.08	.26	0.08	.25	0.21
Adverse family situation	0.00	[-0.02;0.01]	0.33	<.001	0.32	<.001	0.38
Parental education	0.00	[-0.02;0.01]	-0.33	<.001	-0.33	<.001	0.22
Parental psychopathology	0.00	[-0.01;0.03]	0.13	.10	0.13	.09	2.15
Trauma exposure	0.00	[-0.03;0.02]	0.43	<.001	0.42	<.001	0.89
zlogAlpha-Amylase							
Prenatal smoking	0.00	[-0.02;0.02]	0.16	.05	0.16	.05	0.09
Prenatal violence	0.00	[-0.03;0.03]	0.21	.01	0.20	.01	0.73
Maternal age at birth	0.00	[-0.03;0.03]	-0.27	<.001	-0.27	<.001	0.29
Single parenthood	0.00	[-0.04;0.02]	0.14	.08	0.13	.08	2.49
Early insufficient care	0.00	[-0.02;0.02]	0.08	.25	0.08	.25	0.71
Adverse family situation	0.00	[-0.02;0.01]	0.33	<.001	0.32	<.001	0.83
Parental education	0.01	[-0.02;0.03]	-0.34	<.001	-0.33	<.001	1.57
Parental psychopathology	0.00	[-0.03;0.03]	0.13	.10	0.13	.09	1.55
Trauma exposure	0.00	[-0.02;0.01]	0.42	<.001	0.42	<.001	0.01
zlogDHEA-S							
Prenatal smoking	0.00	[-0.02;0.03]	0.15	.05	0.16	.05	2.31
Prenatal violence	0.00	[-0.02;0.03]	0.20	.01	0.20	.01	1.34
Maternal age at birth	-0.01	[-0.03;0.01]	-0.26	<.01	-0.27	<.01	2.24
Single parenthood	-0.00	[-0.03;0.01]	0.13	.08	0.13	.08	3.23
Early insufficient care	-0.00	[-0.02;0.01]	0.08	.23	0.08	.25	2.05
Adverse family situation	0.00	[-0.01;0.03]	0.32	<.001	0.32	<.001	1.29
Parental education	-0.00	[-0.03;0.01]	-0.33	<.001	-0.33	<.001	1.04
Parental psychopathology	-0.00	[-0.03;0.03]	0.14	.08	0.13	.09	0.67
Trauma exposure	0.01	[-0.01;0.03]	0.41	<.001	0.42	<.001	1.53
zlogEstradiol							
Prenatal smoking	0.01	[-0.01;0.05]	0.15	.06	0.16	.05	7.67
Prenatal violence	0.02	[-0.01;0.05]	0.19	.02	0.20	.01	8.57
Maternal age at birth	0.00	[-0.03;0.02]	- -0.27	<.001	-0.27	<.001	1.64
Single parenthood	0.02	[-0.01;0.06]	0.11	.16	0.13	.08	17.03
Early insufficient care	0.02	[-0.01;0.06]	0.05	.47	0.08	.25	24.92
Adverse family situation	0.01	[-0.01;0.04]	0.32	<.001	0.32	<.001	3.89
Parental education	-0.02	[-0.06;0.00]	--0.31	<.001	-0.33	<.001	5.85
Parental psychopathology	-0.01	[-0.04;0.03]	0.14	.09	0.13	.09	4.73
Trauma exposure	0.00	[-0.02;0.03]	0.43	<.001	0.42	<.001	0.99

	Indirect effect	*95% CI [LB;UB]*	Direct effect	*p*	Total effect	*p*	Proportion of total effect mediated [%]
FEMALES							
zlogProgesterone							
Prenatal smoking	0.00	[-0.02;0.02]	0.16	.05	0.16	.05	1.10
Prenatal violence	0.01	[-0.01;0.03]	0.20	.01	0.20	.01	3.75
Maternal age at birth	-0.00	[-0.02;0.02]	-0.27	<.01	-0.27	<.01	0.45
Single parenthood	0.01	[-0.01;0.05]	0.12	.14	0.13	.08	10.13
Early insufficient care	0.01	[-0.01;0.04]	0.07	.34	0.08	.25	14.47
Adverse family situation	0.01	[-0.02;0.03]	0.32	<.001	0.32	<.001	1.71
Parental education	-0.00	[-0.03;0.02]	-0.33	<.001	-0.33	<.001	1.43
Parental psychopathology	-0.01	[-0.04;0.01]	0.14	.08	0.13	.09	5.12
Trauma exposure	-0.00	[-0.02;0.02]	0.43	<.001	0.42	<.001	0.61
zlogOxytocin		-					
Prenatal smoking	0.00	[-0.03;0.02]	0.16	.04	0.16	.05	0.72
Prenatal violence	0.00	[-0.03;0.01]	0.21	.01	0.20	.01	2.26
Maternal age at birth	0.01	[-0.01;0.03] -	-0.28	<.001	-0.27	<.001	3.34
Single parenthood	0.00	[-0.02;0.02]	0.14	.08	0.13	.08	1.08
Early insufficient care	-0.01	[-0.05;0.02]	0.09	.22	0.08	.25	12.15
Adverse family situation	0.00	[-0.02;0.03]	0.32	<.001	0.32	<.001	0.76
Parental education	0.00	[-0.02;0.01]	-0.33	<.001	-0.33	<.001	0.42
Parental psychopathology	-0.01	[-0.04;0.01]	0.14	.07	0.13	.09	6.96
Trauma exposure	0.00	[-0.03;0.02]	0.42	<.001	0.42	<.001	0.58
MALES							
zlogCortisol							
Prenatal smoking	0.00	[-0.03;0.04]	0.33	<.001	0.33	<.001	0.04
Prenatal violence	0.00	[-0.02;0.03]	0.35	<.001	0.35	<.001	0.16
Maternal age at birth	0.00	[-0.03;0.02]	-0.25	.01	-0.25	.01	0.13
Single parenthood	0.00	[-0.03;0.03]	0.35	<.001	0.35	<.001	0.04
Early insufficient care	-0.01	[-0.03;0.02]	0.27	.02	0.27	.02	2.20
Adverse family situation	0.00	[-0.02;0.04]	0.41	<.001	0.41	<.001	0.45
Parental education	0.00	[-0.04;0.04]	-0.37	<.001	-0.37	<.001	0.16
Parental psychopathology	0.00	[-0.03;0.03]	0.18	.09	0.18	.09	0.53
Trauma exposure	0.00	[-0.02;0.04]	0.34	<.001	0.34	<.001	0.29
zlogTestosterone							
Prenatal smoking	0.01	[-0.03;0.04]	0.32	<.001	0.33	<.001	1.88
Prenatal violence	0.00	[-0.01;0.02]	0.34	<.001	0.35	<.001	0.87
Maternal age at birth	0.01	[-0.01;0.05]	-0.25	.01	-0.25	.01	3.76
Single parenthood	0.00	[-0.03;0.03]	0.35	<.001	0.35	<.001	0.49
Early insufficient care	-0.01	[-0.04;0.01]	0.27	.02	0.27	.02	2.70
Adverse family situation	0.01	[-0.03;0.05]	0.40	<.001	0.41	<.001	2.17
Parental education	0.00	[-0.02;0.03]	-0.36	<.001	-0.37	<.001	0.10
Parental psychopathology	0.01	[-0.02;0.05]	0.17	.11	0.18	.09	4.70
Trauma exposure	0.00	[-0.01;0.03]	0.34	<.001	0.34	<.001	0.84
zlogDHEA-S							

	Indirect effect	*95% CI [LB;UB]*	Direct effect	*p*	Total effect	*p*	Proportion of total effect mediated [%]
MALES							
zlogDHEA-S							
Prenatal smoking	0.01	[-0.01;0.07]	0.31	<.001	0.33	<.001	4.06
Prenatal violence	0.01	[-0.01;0.06]	0.33	<.001	0.35	<.001	3.57
Maternal age at birth	0.00	[-0.03;0.04]	-0.25	.01	-0.25	.01	1.65
Single parenthood	0.01	[-0.02;0.05]	0.34	<.001	0.35	<.001	2.37
Early insufficient care	0.00	[-0.02;0.04]	0.26	.03	0.27	.02	1.67
Adverse family situation	0.01	[-0.02;0.06]	0.40	<.001	0.41	<.001	2.13
Parental education	-0.01	[-0.06;0.02]	-0.35	<.001	-0.37	<.001	3.44
Parental psychopathology	0.00	[-0.04;0.06]	0.17	.10	0.18	.09	0.12
Trauma exposure	-0.01	[-0.05;0.03]	0.34	<.001	0.34	<.001	1.93
zlogEstradiol							
Prenatal smoking	0.01	[-0.01;0.05]	0.32	<.001	0.33	<.001	3.32
Prenatal violence	0.00	[-0.02;0.03]	0.34	<.01	0.35	<.001	0.61
Maternal age at birth	-0.01	[-0.05;0.02]	-0.24	.02	-0.25	.01	4.03
Single parenthood	0.01	[-0.01;0.06]	0.34	<.01	0.35	<.01	2.85
Early insufficient care	0.02	[-0.02;0.07]	0.25	.04	0.27	.02	6.20
Adverse family situation	-0.01	[-0.05;0.03]	0.42	<.001	0.41	<.001	1.40
Parental education	-0.00	[-0.05;0.03]	-0.37	<.01	-0.37	<.01	0.99
Parental psychopathology	-0.02	[-0.07;0.01]	0.20	.05	0.18	.09	11.24
Trauma exposure	-0.01	[-0.06;0.02]	0.36	<.01	0.34	<.01	3.71
zlogProgesterone							
Prenatal smoking	-0.00	[-0.03;0.02]	0.33	<.001	0.33	<.001	0.45
Prenatal violence	-0.00	[-0.03;0.02]	0.35	<.01	0.35	<.01	0.20
Maternal age at birth	-0.00	[-0.03;0.02]	-0.25	0.01	-0.25	.01	0.86
Single parenthood	-0.00	[-0.04;0.03]	0.35	<.01	0.35	<.01	0.79
Early insufficient care	0.01	[-0.02;0.04]	0.26	.03	0.27	.02	2.24
Adverse family situation	-0.00	[-0.03;0.02]	0.42	<.001	0.41	<.001	0.45
Parental education	0.01	[-0.01;0.07]	-0.38	<.001	-0.37	<.01	3.59
Parental psychopathology	-0.00	[-0.03;0.02]	0.18	.08	0.18	.09	1.71
Trauma exposure	-0.01	[-0.05;0.01]	0.36	<.01	0.34	<.01	4.00

Note. The standardized estimates of the effects are reported. For details on mediation analyses and effects, see *Statistical Analyses* in the main text. CI=confidence interval, DHEA-S=dehydroepiandrosterone-sulfate, LB=lower bound, UB=upper bound, zlog=z-standardized and log-transformed.

References

1. Kaufman J, Birmaher B, Brent D, et al. Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. *J Am Acad Child Adolesc Psychiatry*. 1997;36(7):980-988. doi:10.1097/00004583-199707000-00021
2. Wechsler D. *Wechsler Intelligence Scale for Children—Fourth Edition (WISC-IV)*. San Antonio: TX: Psychological Corporation; 2003.
3. Wechsler D. *Wechsler Adult Intelligence Scale–Fourth Edition (WAIS–IV)*. San Antonio: TX: NCS Pearson 22; 2008.
4. Wechsler D. *Wechsler Abbreviated Scale of Intelligence.* San Antonio: TX: Psychological Corporation; 1999.
5. Freitag CM, Hänig S, Schneider A, et al. Biological and psychosocial environmental risk factors influence symptom severity and psychiatric comorbidity in children with ADHD. *J Neural Transm*. 2012;119:81-94.
6. Boden JM, Fergusson DM, Horwood LJ. Risk factors for conduct disorder and oppositional/defiant disorder: Evidence from a New Zealand birth cohort. *J Am Acad Child Adolesc Psychiatry*. 2010;49(11):1125-1133. doi:10.1016/j.jaac.2010.08.005
7. Organisation for Economic Co-operation and Development. *Classifying Educational Programmes: Manual for ISCED-97 Implementation in OECD Countries*. (OECD, ed.). Paris; 1999.