Sex-specific associations between maternal prenatal cortisol and child callous-unemotional traits:

Findings from the Wirral Child Health and Development Study

Running title: Prenatal cortisol and callous-unemotional traits

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Keywords: cortisol; pregnancy; HPA axis; callous-unemotional traits; internalizing symptoms; fetal programming

Abstract

Background: Maternal prenatal stress may impact on fetal development in a sex-dependent way. Evidence from the human and animal literature supports a theory of glucocorticoid-mediated risk in females, which results in elevated affective symptoms via increased amygdala connectivity and activity. We sought to investigate whether this mechanism may be protective against psychopathology associated with reduced amygdala activity, specifically callous-unemotional (CU) traits.

Methods: Participants (n=225) were members of a stratified subsample of an epidemiological cohort (n=1233). Maternal diurnal cortisol was measured using the log of the area under the curve (LogAUC) from saliva samples collected over two days at 32 weeks gestation (on waking, 30-min post-waking and during the evening). Mothers reported on child CU traits and internalizing symptoms at 2.5, 3.5 and 5 years of age.

Results: Higher maternal prenatal cortisol was significantly associated with lower CU traits in girls. This effect remained when controlling for confounders and internalizing symptoms. The effect in boys was consistently different from girls (interaction term $p < 0.001$), but never different from zero, indicating no association. There was a significant interaction term to predict internalizing symptoms, however the effect became non-significant when confounders and CU traits were included in the model.

Conclusions: The findings extend existing understanding of the adaptive model of prenatal programming in females, where exposure to prenatal stress increases risk for affective disorders via glucocorticoid mechanisms and altered amygdala functioning. Our findings suggest that exposure to elevated prenatal cortisol in females may confer risk or be a protective mechanism depending on the outcome.

Accumulating evidence from human and animal research suggests that fetal conditions may be associated with offspring development in a sex-dependent way. Of relevance to this paper, glucocorticoid-mediated mechanisms associated with increased emotional reactivity, internalizing symptoms and later depressive/anxious symptoms in adolescence may be specific to females. Increased amygdala activation has been implicated in this process. By contrast reduced amygdala activation is associated with callous-unemotional (CU) traits raising the question of whether this mechanism may also be protective against the development of CU traits also in a sex-dependent manner. In this study we tested the hypothesis that elevated prenatal maternal cortisol is associated with lower CU traits in girls only.

Sex differences in psychopathology following exposure to prenatal stress have been described in both the human and animal literature. Female (but not male) rats exposed to *in utero* gestational stress exhibit elevated anxiety and depression-like behaviours (1), such as reduced explorations of open arms in the elevated maze test (2), and an increased length of immobility in the forced swim test (3). The effects of prenatal stress on female offspring depression-like behaviour are eliminated by adrenalectomy (2), consistent with risks mediated by glucocorticoid mechanisms that are specific to females. In the human literature, maternal prenatal cortisol, the main glucocorticoid hormone, has been associated with the following outcomes in females, but not males: reduced fetal growth, an established risk factor for later depression (4); increased infant negative emotionality (5, 6); fearful infant temperament (7); affective problems in childhood (8); pre-adolescent anxiety (7), depression and a flattened diurnal cortisol profile in adolescence (9). In combination, these results present convincing evidence that exposure to prenatal cortisol increases risk of later affective symptoms in females.

There is also evidence from both the human and animal literature that the amygdala is implicated in neural mechanisms mediating associations between prenatal glucocorticoids and affective disorders (8). The amygdala develops during an early embryonic stage (10), contains a high concentration of glucocorticoid receptors (11), and is thus likely susceptible to varying concentrations

of maternal glucocorticoids. Animal models show that *in utero* exposure to elevated maternal glucocorticoids is associated with increased glucocorticoid receptor expression in the amygdala (12), structural changes in sub regions of amygdaloid nuclei (13) and associated depression-like behaviours in adult offspring (12). Human studies have demonstrated a female-specific association between prenatal stress and negative emotionality (5, 6) and fearful temperament (7), which have been linked with amygdala activation. There is also direct evidence that elevated maternal cortisol in early, but not late, pregnancy is associated with a larger right amygdala volume in girls, but not boys (8). In the same study, high prenatal cortisol was also positively associated with affective problems in girls, an effect which was, in part, mediated by right amygdala volume (8) and increased amygdala connectivity (14) during infancy. Associations, in girls and not in boys, between prenatal maternal cortisol and brain network properties have been demonstrated, with indications that these were associated with increased internalizing symptoms (15). Thus there is emerging evidence for female specific pathways linking prenatal cortisol exposure to altered brain structure and risk for child symptoms.

While amygdala activation is associated with increased internalizing symptoms it is also associated with many important adaptive functions including empathy (16). Reduced empathy is thought to be a core component of callous unemotional (CU) traits, raising the question of whether prenatal cortisol is associated with lower CU traits, also in a sex-dependent manner. CU traits are characterised by a lack of concern for others' feelings, particularly distress, a lack of guilt and diminished emotional expression (17) and have been shown to delineate a subgroup of children with conduct problems who show more severe and persistent antisocial behaviour (18). Evidence from human imaging studies show that individuals with high CU traits have reduced connectivity between the amygdala and prefrontal cortex (19, 20), and adults with psychopathic traits have lower amygdala volume (21). Further, individuals who exhibit high levels of CU traits demonstrate lower right amygdala activity in response to viewing fearful faces (20, 22) and participating in theory of mind tasks (23). There is also evidence that reduced amygdala responses to a face-emotion processing task

mediates the relationship between CU traits and proactive aggression (24). Thus, this literature describes a key role of amygdala structure, connectivity and function in CU traits. We therefore predicted that elevated maternal cortisol will be associated with lower CU traits. In view of the evidence for sex-dependent glucocorticoid mechanisms linking fetal conditions to child outcomes we predicted that this would be seen in girls only. In the light of the reported associations between maternal cortisol and child internalizing symptoms we also predicted that elevated maternal cortisol would be associated with higher levels of internalizing symptoms, and only in girls.

Methods and Materials

Design

Participants were members of the Wirral Child Health and Development Study (WCHADS), a prospective epidemiological study starting in pregnancy. All women gave written informed consent at the point of recruitment in the antenatal clinic and at each subsequent assessment phase. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. All procedures involving human subjects/patients were approved by the Cheshire North and West Research Ethics committee on the 27th June 2006 (pregnancy to age 1 waves) and 7th June 2010 (age 2.5 to 5 year waves). The study used a two stage stratified design in which a consecutive general population sample (the ‘extensive’ sample) is used to generate a smaller ‘intensive’ sample stratified by psychosocial risk with more detailed measurement over time and both are followed in tandem (see Sharp et al [25] for a detailed description of the sample). The sample stratification has been described previously (25) but in brief, the stratification was based on maternal responses to questions about psychological abuse in their current or recent partner relationship (26). The stratification variable was chosen for its known association with a variety of risk factors for early child development.

Sample

The whole cohort (extensive sample) comprised 1,233 women recruited in pregnancy with a live, singleton baby for long-term follow up post-birth. The mean age at recruitment was 26.8 years (SD = 5.8, range 18-51), 41.8% of the sample were in the most deprived quintile of UK neighbourhoods (23) and 96.1% were White British. This report uses the stratified subsample of mothers recruited to the intensive sample at 32 weeks pregnancy and available at birth for longitudinal follow-up (n=316). Diurnal saliva samples were collected at 32 weeks gestation and were available for 239 of this subsample of mothers. Complete and usable cortisol data was provided by 225 mothers who form the sample used in this analysis; their demographic characteristics are presented in Table 1. Mothers reported on child behaviours and CU traits and their own depression symptoms when the children were aged 2.5 (mean age: 30.98 months, SD = 2.34), 3.5 (mean age: 41.78 months, SD = 1.96) and 5 years (mean age: 57.82, SD = 2.94). All data analyses were weighted to give estimated means and coefficients for the whole cohort of 1233.

Measures

Maternal cortisol. At 32 weeks gestation, mothers collected saliva samples at home over two consecutive working days. Saliva was collected on waking, 30 min post-waking, and during the evening (approx. 12 h after waking (mean=12h 10min, SD=1h 15min)). Participants stored the samples in their freezer until a research assistant collected them 1-2 weeks later. Samples were stored at -20°C before transportation to Imperial College London on dry ice for analysis. All samples were assayed for salivary cortisol using a commercially available immunoassay (Salimetrics, UK). Inter- and intra-assay variation were 7.9% and 8.9% respectively. Salivary assays were run in duplicate except for a small minority of cases with minimal volume (n=3). In this study we used the log of the area under the curve (LogAUC) as an index of diurnal cortisol release. The area under the curve was calculated using the trapezoid method with respect to ground, taking the mean (over 2 days) of the awakening, 30 minutes and 12 hour post-awakening measures. A full set of maternal saliva samples

over two days was available for 229 participants. Of these, five cases were unusable due to time of collection not being recorded or researcher labelling error, resulting in a sample of 225. Ten participants provided saliva samples over one day, and their raw scores were used in analyses instead of a mean score. Cortisol measured across the two days was correlated at the three time-points (waking cortisol $r=0.485$, $p<0.001$, 30-min post-waking $r=0.473$, $p<0.001$, 12-h post-waking $r=0.157$, $p=0.02$).

Child callous-unemotional (CU) traits. CU traits were assessed by mother-report at 2.5, 3.5 and 5 years. There is evidence that CU traits can be validly measured in preschool aged children, with a recent meta-analysis of 10 studies of children aged <5 years demonstrating that CU traits predict later problem behaviour over and above earlier problem behaviour (28). In this study, CU traits were assessed using a combination of the Antisocial Processes Screening Device (29) and items from the Child Behaviour Checklist (CBCL; (30)), the Brief Infant Toddler Assessment (BITSEA; (31)) and the Strengths and Difficulties Questionnaire (SDQ; (32)). We have previously created CU traits latent factor scores at 2.5, 3.5 and 5 years (33) by subjecting items to exploratory and confirmatory factor analyses in MPlus (34). The items at each age were allowed to vary to reflect developmental differences in the manifestation of CU traits. For this analysis, a latent variable was created from the factor scores from the three age time points to represent CU traits from 2.5 to 5 years. The summary statistics for this variable, and the associations between the three age points have been published previously (31).

Child internalizing symptoms. Maternal reports of child internalizing symptoms were assessed at 2.5, 3.5 and 5 years using the Child Behaviour Checklist (CBCL; (28)). This measure has been used extensively in studies of child and adolescent emotional and behavioural disorders. The scale had 99 items, each scored 0 (not true), 1 (somewhat or sometimes true) and 2 (very true or often true), which are summed to create seven syndrome scales and two broadband internalizing and externalising subscales. The total scores on the internalizing symptoms scale were used in this analysis: a latent

variable was created from the square-root of the internalizing symptom scale at each time point as a way of capturing long-term/persistent individual differences.

Confounders. A number of potential confounding variables were included in the analysis to account for socio-demographic risk. The variables were selected because they have been shown to be associated with child behavioural problems in previous research. This included mothers' age at conception (0 => age 20 years, 1 = age 21 to 30 years, 2=> age 31 years), mothers' age at leaving education (0 = age 18 or younger, 1 = age 19 years or older), marital status (0 = single or partner living elsewhere, 1 = married or cohabiting), smoking during pregnancy (0 = no smoking, 1= smoking) and deprivation. Deprivation was assessed using the revised English Index of Multiple Deprivation (IMD); (27) based on data collected from the UK Census in 2001. According to this system, postcode areas in England are ranked from most deprived (i.e. IMD of 1) to least deprived (i.e. IMD of 32,482) based on deprivation in seven domains: income, employment, health, education and training, barriers to housing and services, living environment, and crime. All mothers were given IMD ranks according to the postcode of the area where they lived and assigned to a quintile based on the UK distribution of deprivation. A binary variable, with 1= most deprived quintile versus 0 = all other quintiles, was used for analysis. Prenatal depression and anxiety reported at the time of the cortisol assessment (32 weeks) were also included as covariates; depression was measured using the Edinburgh Postnatal Depression Scale (EPDS; (35)) and anxiety was measured using the Spielberger State-Trait Anxiety Inventory, (STAI; (36)). Mothers' depression at the time of reporting child CU traits and internalizing problems was included as a covariate to account for any potential bias on reporting. At ages 2.5 and 3.5 years maternal depression was assessed using the EPDS and at age 5 years maternal depression was assessed using the Centre for Epidemiological Studies - Depression (CES-D; (37)). A single latent variable was created from the three time points for analysis.

Statistical analyses

All analyses were undertaken in Stata version 14, primarily using the `sem` command for structural equation models (SEM). The two-phase stratified sample design allows estimates to be reported for the general population by applying inverse probability weights. These weights were constructed to take account of the sample design stratification factor and additional variables associated with attrition and response on the variables required for the analysis: maternal age, depression, and smoking in pregnancy, years of education, marital status and the deprivation index for the mother's home neighbourhood. The weights compensate for the systematic differences between our analysed sample and the whole population cohort. Modelled as latent variables in the SEMs (see Figure 1) using the `mlmv` estimator, measures of child CU traits and internalizing problems were not required to be complete, a partial set of measures being sufficient for inclusion in the model. The CBCL internalizing scores were square-root transformed to achieve approximate normality. Test statistics and confidence intervals for weighted means, and coefficient estimates, were based on survey adjusted Wald tests (t-tests if single degrees of freedom (df) or F-tests if multiple df) using the robust 'sandwich' estimator of the parameter covariance matrix (38). All models included the paths corresponding to the main effects of sex, maternal prenatal cortisol and the interaction between maternal prenatal cortisol and sex shown in Figure 1. Model 1 included no confounders; model 2 included confounders (detailed below) excluding the maternal postnatal depression score; model 3 included confounders and maternal depression. Two versions of Model 4 were estimated, Model 4a included an additional effect from the child internalizing factor to the CU traits factor, and model 4b instead included the effect from the child CU traits to the internalizing factor. These last two models were to clarify the specificity of effects, i.e. to test effects of maternal cortisol and gender on one factor while accounting for possible effects from the other.

The following confounders were assessed during pregnancy were included in models 2, 3 and 4: maternal education, marital status, deprivation, prenatal smoking, prenatal depression and anxiety. A

factor constructed from measures of maternal depression when the child was 2.5, 3.5 and 5 years was also included as a confounder in models 3 and 4.

Results

Descriptive statistics for all measures used in the analysis are displayed in Table 1. These showed very similar scores for boys and girls in the exposures and in the average behavioural outcomes. For ages 2.5 to 3.5 years, and 2.5 to 5 years and 3.5 to 5 years, the correlations among the transformed CBCL internalizing scores were 0.70, 0.59 and 0.65 and for the CU traits factor scores, 0.63, 0.52 and 0.61.

Table 2 displays the results of the structural equation models predicting child CU traits and child internalizing problems from maternal prenatal cortisol and child sex. In the simple model without confounders (Model 1) there were significant effects of child sex, maternal prenatal cortisol, and a sex by cortisol interaction on child CU traits. A similar pattern of significant effects was also shown for Model 1 in the prediction of child internalizing problems. The main effect for cortisol corresponds to the estimated effect for girls, while that for boys is the sum of the effect for girls and the cortisol by gender interaction term. Testing these summed coefficients indicated that for boys neither CU traits nor internalizing problems were significantly associated with maternal cortisol ($p=.719$ and $.274$ respectively). However for girls, increased maternal cortisol was associated with both lower CU traits and lower internalizing problems. The (unweighted) pattern of effects are shown in Figure 2.

The addition of confounders (sample stratifier, maternal age, maternal education, smoking in pregnancy, mother not living with partner, neighbourhood deprivation, prenatal maternal anxiety and depression scores) in Model 2 had little impact for the maternal cortisol and gender effects on child CU traits, but a more substantial impact on their effects on child internalizing problems, these now all becoming smaller and non-significant. In Model 3 maternal depression at the times of reporting

the outcomes was added as a further confound to account for potential mood biasing effects on reporting. Maternal depression significantly predicted higher child CU traits but again the pattern and significance of the sex and cortisol effects were unchanged. Maternal depression also significantly predicted higher child internalizing problems, but the effects of child sex and prenatal cortisol were further reduced and remained non-significant.

In Model 4a a path was added from the internalizing factor to the CU traits factor to test the effect of maternal cortisol and sex on child CU traits after accounting for internalizing problems. Whilst the internalizing factor strongly predicted CU traits, the estimated effects of cortisol and sex on CU traits retained the same pattern, somewhat reduced in magnitude but still clearly significant. Maternal depression at the time of reporting no longer predicted CU traits after the addition of this path. In Model 4b a path was added from the CU traits factor to the internalizing problems factor. This additional path was strongly significant and reduced the effect of maternal depression on internalizing problems, further, it essentially removed what remained of the cortisol-gender effects on the internalizing factor.

Fitting the simple model to boys and girls separately suggested that for boys 1% of the CU traits factor and 2% of the internalizing problems factor was explained by maternal cortisol, while for girls the estimates were 26% and 7% respectively. When all confounders and an effect from the internalizing factor were allowed for, cortisol still explained 18% of the variance of the CU traits factor. However, accounting for confounders and the effects of the CU factor on the internalizing problems factor reduced the variance of the internalizing factor attributable to maternal cortisol to 0%.

Discussion

Based on recent evidence for sex-dependent associations between prenatal maternal cortisol and amygdala function (14), and known associations between amygdala under-activity and CU traits, we

predicted that elevated prenatal cortisol would be associated with reduced CU traits and increased internalizing problems only in girls. Using data from a prospective, longitudinal cohort stratified by risk, we examined sex-specific associations between maternal prenatal cortisol at 32 weeks gestation and child CU traits assessed at three time points from ages 2.5 to 5.0 years. There was a negative association between maternal prenatal cortisol and CU traits, indicating that higher prenatal cortisol was associated with lower CU traits, moderated by sex of child. Higher maternal cortisol was associated with lower CU traits only in girls. This effect was evident when controlling for relevant confounding variables, and when controlling for internalizing problems. Contrary to our prediction, higher maternal prenatal cortisol, sex and their interaction were also associated with lower internalizing problems, however this effect was entirely explained by the positive association between CU traits and internalizing problems.

Our findings are consistent with an accumulating literature that supports the existence of sex-dependent glucocorticoid mechanisms for early child behaviours. Previously, the focus has been on the implications of increased negative emotionality (5, 6), fearfulness (7), anxiety and depression (7, 9), with indications of mediation by the amygdala (8) in girls, but not boys. Here we show, consistent with evidence that decreased amygdala activity is associated with elevated CU traits, that increased maternal cortisol in pregnancy confers a protection against the development of CU traits in girls.

While there have been some indications from previous research of sex differences in the origins of CU traits these have not been consistent, and recent reviews have highlighted the need for more work on this topic (39). For example, Fontaine et al. (40) found sex differences in MZ-DZ concordance for estimated trajectories of CU traits at age 7, 9 and 12 years. In boys there was a strong genetic effect, but in girls shared environment made the major contribution. By contrast, in a smaller twin sample of 10 year olds, a direct test of sex differences found no support for differences between boys and girls in genetic effects on CU traits. Similarly a recent study reported an association between increased anterior insula volume and CU traits in boys only (41), in contrast to previous studies suggesting possible associations between decreased anterior insula function and volume and

antisocial behaviour problems (42, 43). Marked differences in sampling and measurement across the studies limits the scope to evaluate these inconsistencies.

A significant strength of this study is the epidemiological sample recruited during pregnancy with a subsample stratified by psychosocial risk for more detailed assessment. This enabled data from the time consuming and costly measures of maternal prenatal cortisol to be weighted back to the general population. Our measures were assessed prospectively and included relevant prenatal confounding variables, and measures of maternal mood that might have biased maternal reporting of CU traits and internalizing problems. We took a latent variable approach to modelling the CU traits outcome by combining reports from three time points throughout early childhood. This increased the robustness of the results by reducing the influence of measurement error. It also allowed us to examine a CU traits outcome that reflected persistence of CU traits, likely to be associated with poorer outcomes later in childhood. Limitations of the study include that participants self-reported the timing of the cortisol sample collection, which could potentially be inaccurate and introduce error. We also had no information on time spent asleep before the first morning sample, or information on the time of the last meal/drink before each cortisol sample, which could affect the cortisol measurement (44, 45).

The findings raise questions and provide indicators for future investigation of glucocorticoid mechanisms in generating on the one hand risk for internalizing problems, and on the other hand protective prosocial or empathic responses particularly in females. There is some evidence that these may be two sides of the same coin. For example daughters, but not sons, of mothers with postnatal depression showed greater portrayal of prosocial behaviours in their doll play at age 5, which mediated the association between postnatal depression and adolescent depression (46). On the other hand in this study CU traits and internalizing problems were positively correlated, consistent with findings from other general population studies (47), but not predicted if the same mechanism underpins increased internalizing problems and increased empathy. Recent evidence suggests that some individuals with CU traits or adult psychopathy also show elevated anxiety and that the developmental origins for CU traits with and without anxiety may differ (48). Thus it may be that

the neurobiology of CU traits, and in particular the role of amygdala function, differs depending on the level of associated anxiety. While our prediction for this study was based on findings of amygdala function in relation to prenatal cortisol levels and in relation to CU traits, we did not measure amygdala function and it cannot be assumed that this was the mediator of the association reported here. Nevertheless, our findings together with those showing the same pattern of sex-dependent associations between prenatal cortisol and elevated infant negative emotionality and child internalizing problems (5, 6, 14), imply the need for further work regarding the circumstances under which prenatal glucocorticoid and postnatal amygdala activity mechanisms are associated on the one hand with increased emotionality, and on the other hand with increased responsiveness to others' negative emotions.

Acknowledgments

The research was funded by grants from the UK Medical Research Council (G0900654; G0400577; MR/L022257/1). The authors are grateful to all participating families and research staff who contributed to this work. They also thank Wirral University Teaching Hospital NHS Foundation Trust, Wirral Community NHS Foundation Trust, Cheshire and Wirral Partnership NHS Foundation Trust and the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust. The authors have declared no competing or potential conflicts of interest.

Disclosures

Dr Wright, Professor Pickles, Dr Braithwaite, Professor Sharp and Professor Hill reported no financial interest or potential conflict of interest.

References

1. Schulz KM, Pearson JN, Neeley EW, Berger R, Leonard S, Adams CE, et al. Maternal stress during pregnancy causes sex-specific alterations in offspring memory performance, social interactions, indices of anxiety, and body mass. *Physiol Behav* 2011;104(2):340-7.
2. Zagron G, Weinstock M. Maternal adrenal hormone secretion mediates behavioural alterations induced by prenatal stress in male and female rats. *Behav Brain Res* 2006;175(2):323-8.
3. Frye CA, Wawrzycki J. Effect of prenatal stress and gonadal hormone condition on depressive behaviors of female and male rats. *Horm Behav* 2003;44(4):319-26.
4. Braithwaite EC, Hill J, Pickles A, Glover V, O'Donnell K, Sharp H. Associations between maternal prenatal cortisol and fetal growth are specific to infant sex: findings from the Wirral Child Health and Development Study. *J Dev Orig Health Dis* 2018:1-7.
5. Braithwaite EC, Pickles A, Sharp H, Glover V, O'Donnell K, Tibu F, et al. Maternal prenatal cortisol predicts infant negative emotionality in a sex-dependent manner. *Physiol Behav* 2017.
6. Braithwaite EC, Murphy SE, Ramchandani PG, Hill J. Associations between biological markers of prenatal stress and infant negative emotionality are specific to sex. *Psychoneuroendocrinology*. 2017;86:1-7.
7. Sandman CA, Glynn LM, Davis EP. Is there a viability-vulnerability tradeoff? Sex differences in fetal programming. *J Psychosom Res* 2013;75(4):327-35.
8. Buss C, Davis EP, Shahbaba B, Pruessner JC, Head K, Sandman CA. Maternal cortisol over the course of pregnancy and subsequent child amygdala and hippocampus volumes and affective problems. *Proc Natl Acad Sci U S A* 2012;109(20):E1312-9.
9. Van den Bergh BR, Van Calster B, Smits T, Van Huffel S, Lagae L. Antenatal maternal anxiety is related to HPA-axis dysregulation and self-reported depressive symptoms in adolescence: a prospective study on the fetal origins of depressed mood. *Neuropsychopharmacology* 2008;33(3):536-45.

10. Humphrey T. The development of the human amygdala during early embryonic life. *J Comp Neurol* 1968;132(1):135-65.
11. Wang Q, Verweij EW, Krugers HJ, Joels M, Swaab DF, Lucassen PJ. Distribution of the glucocorticoid receptor in the human amygdala; changes in mood disorder patients. *Brain Struct Funct* 2014;219(5):1615-26.
12. Welberg LA, Seckl JR, Holmes MC. Inhibition of 11beta-hydroxysteroid dehydrogenase, the foeto-placental barrier to maternal glucocorticoids, permanently programs amygdala GR mRNA expression and anxiety-like behaviour in the offspring. *Eur J Neurosci* 2000;12(3):1047-54.
13. Salm AK, Pavelko M, Krouse EM, Webster W, Kraszpulski M, Birkle DL. Lateral amygdaloid nucleus expansion in adult rats is associated with exposure to prenatal stress. *Brain Res Dev Brain Res* 2004;148(2):159-67.
14. Graham AM, Rasmussen JM, Entringer S, Ward EB, Rudolph MD, Gilmore JH, et al. Maternal Cortisol Concentrations During Pregnancy and Sex Specific Associations with Neonatal Amygdala Connectivity and Emerging Internalizing Behaviors. 2018. *Biol Psychiatry*.
15. Kim DJ, Davis EP, Sandman CA, Sporns O, O'Donnell BF, Buss C, et al. Prenatal Maternal Cortisol Has Sex-Specific Associations with Child Brain Network Properties. *Cereb Cortex* 2017;27(11):5230-41.
16. Del Casale A, Kotzalidis GD, Rapinesi C, Janiri D, Aragona M, Puzella A, et al. Neural functional correlates of empathic face processing. *Neurosci Lett* 2017;655:68-75.
17. Frick PJ. Extending the construct of psychopathy to youth: implications for understanding, diagnosing, and treating antisocial children and adolescents. *Can J Psychiatry* 2009;54(12):803-12.
18. Frick PJ, Ray JV, Thornton LC, Kahn RE. Can callous-unemotional traits enhance the understanding, diagnosis, and treatment of serious conduct problems in children and adolescents? A comprehensive review. *Psychol Bull* 2014;140(1):1-57.

19. Finger EC, Marsh A, Blair KS, Majestic C, Evangelou I, Gupta K, et al. Impaired functional but preserved structural connectivity in limbic white matter tracts in youth with conduct disorder or oppositional defiant disorder plus psychopathic traits. *Psychiatry Res* 2012;202(3):239-44.
20. Marsh AA, Finger EC, Mitchell DG, Reid ME, Sims C, Kosson DS, et al. Reduced amygdala response to fearful expressions in children and adolescents with callous-unemotional traits and disruptive behavior disorders. *Am J Psychiat* 2008;165(6):712-20.
21. Pardini DA, Raine A, Erickson K, Loeber R. Lower amygdala volume in men is associated with childhood aggression, early psychopathic traits, and future violence. *Biol Psychiatry* 2014;75(1):73-80.
22. Alice P. Jones PD, Kristin R. Laurens PD, Catherine M. Herba PD, Gareth J. Barker PD, Essi Viding PD. Amygdala Hypoactivity to Fearful Faces in Boys With Conduct Problems and Callous-Unemotional Traits. *Am J Psychiat* 2009;166(1):95-102.
23. Sebastian CL, McCrory EJ, Cecil CA, Lockwood PL, De Brito SA, Fontaine NM, et al. Neural responses to affective and cognitive theory of mind in children with conduct problems and varying levels of callous-unemotional traits. *Arch Gen Psychiatry* 2012;69(8):814-22.
24. Lozier LM, Cardinale EM, VanMeter JW, Marsh AA. Mediation of the relationship between callous-unemotional traits and proactive aggression by amygdala response to fear among children with conduct problems. *JAMA Psychiatry* 2014;71(6):627-36.
25. Sharp H, Pickles A, Meaney M, Marshall K, Tibu F, Hill J. Frequency of infant stroking reported by mothers moderates the effect of prenatal depression on infant behavioural and physiological outcomes. *PLoS One* 2012;7(10):e45446.
26. Moffitt TE, Caspi A, Krueger R, Magdol L, Margolin G, Silva P, et al. Do partners agree about abuse in their relationship?: A psychometric evaluation of interpartner agreement. *Psychol Assess* 1997;9(1):47-56.

27. Noble M, Wright G, Dibben C, Smith G, McLennan D, Anttila C, et al. *The English Indices of Deprivation 2004 (revised)*. Report to the Office of the Deputy Prime Minister. . In: Unit NR, editor. London 2004
28. Longman T, Hawes DJ, Kohlhoff J. Callous–unemotional traits as markers for conduct problem severity in early childhood: A meta-analysis. *Child Psychiat Hum Dev*. 2016;47(2):326-34.
29. Frick PJ, Hare RD. *The antisocial process screening device (APSD)*. Toronto, ONMulti-Health Systems2001.
30. Achenbach TM, Ruffle TM. The Child Behavior Checklist and related forms for assessing behavioral/emotional problems and competencies. *Pediatr Rev* 2000;21(8):265-71.
31. Briggs-Gowan MJ, Carter AS, Irwin JR, Wachtel K, Cicchetti DV. The Brief Infant-Toddler Social and Emotional Assessment: screening for social-emotional problems and delays in competence. *J Pediatr Psychol* 2004;29(2):143-55.
32. Goodman R. The Strengths and Difficulties Questionnaire: a research note. *J Child Psychol Psychiatry* 1997;38(5):581-6.
33. Wright N, Hill J, Sharp H, Pickles A. Maternal sensitivity to distress, attachment and the development of callous-unemotional traits in young children. *J Child Psychol Psychiatry* 2018.
34. Muthén LK, Muthén BO. *Mplus user's guide*. 7th edn ed. Los Angeles, CA: Muthén & Muthén; 2012.
35. Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. *Br J Psychiat* 1987;150(6):782-6.
36. Spielberger CD, Gorsuch R, L., Lushene P, R., Vagg P, R., Jacobs G, A. *Manual for the State-Trait Anxiety Inventory*: Consulting Psychologists Press, Inc.; 1983.
37. Radloff LS. The CES-D Scale:A Self-Report Depression Scale for Research in the General Population. *Appl Psychol Meas* 1977;1(3):385-401.
38. Binder DA. On the variances of asymptotically normal estimators from complex surveys. *Int Stat Rev* 1983;51:279-92.

39. Viding E, McCrory EJ. Genetic and neurocognitive contributions to the development of psychopathy. *Dev Psychopathol* 2012;24(3):969-83.
40. Fontaine NM, Rijdsdijk FV, McCrory EJ, Viding E. Etiology of different developmental trajectories of callous-unemotional traits. *J Am Acad Child Adolesc Psychiatry* 2010;49(7):656-64.
41. Raschle NM, Menks WM, Fehlbauer LV, Steppan M, Smaragdi A, Gonzalez-Madruga K, et al. Callous-unemotional traits and brain structure: Sex-specific effects in anterior insula of typically-developing youths. *NeuroImage Clin* 2018;17:856-64.
42. Lockwood PL, Sebastian CL, McCrory EJ, Hyde ZH, Gu X, De Brito SA, et al. Association of callous traits with reduced neural response to others' pain in children with conduct problems. *Curr Biol* 2013;23(10):901-5.
43. Rogers JC, De Brito SA. Cortical and Subcortical Gray Matter Volume in Youths With Conduct Problems: A Meta-analysis. *JAMA Psychiatry* 2016;73(1):64-72.
44. Elder GJ, Wetherell MA, Barclay NL, Ellis JG. The cortisol awakening response--applications and implications for sleep medicine. *Sleep Med Rev* 2014;18(3):215-24.
45. Clow A, Thorn L, Evans P, Hucklebridge F. The awakening cortisol response: methodological issues and significance. *Stress* 2004;7(1):29-37.
46. Murray L, Halligan SL, Adams G, Patterson P, & Goodyer IM. Socioemotional development in adolescents at risk for depression: the role of maternal depression and attachment style. *Dev Psychopathology* 2006;18(2):489-516.
47. Barker ED, Salekin RT. Irritable oppositional defiance and callous unemotional traits: is the association partially explained by peer victimization? *J Child Psychol Psychiatry* 2012;53(11):1167-48.
48. Meehan AJ, Maughan B, Cecil CAM, Barker ED. Interpersonal callousness and co-occurring anxiety: Developmental validity of an adolescent taxonomy. *J Abnorm Psychol* 2017;126(2):225-36.

Figure 1. Schematic diagram of structural equation model using maternal prenatal cortisol, infant sex and covariates to predict the CU traits and internalizing behaviour factors. Standardised estimates generated form this model are displayed in Table 2.

Figure 2. Sex-specific associations between maternal prenatal cortisol and the CU traits factor score and internalizing factor score. The figure displays unweighted regression lines with 95% confidence intervals.

Table 1. Demographic characteristics of the sample and descriptive statistics for key study variables by sex.

Variable	N	Female mean	SD	N	Male mean	SD
Maternal cortisol log mean AUC	120	1.12	0.39	105	1.15	0.45
Age 2.5 years CBCL Internalizing total score	104	2.06	1.07	91	2.10	1.09
Age 3.5 years CBCL Internalizing total score	109	2.28	1.11	96	2.25	1.10
Age 5 years CBCL Internalizing total score	107	2.34	1.06	95	2.42	1.26
Age 2.5 years CU factor score	103	-0.07	0.46	91	0.07	0.50
Age 3.5 years CU factor score	104	0.01	0.75	94	0.07	0.75
Age 5 years CU factor score	109	0.05	0.80	95	0.01	0.91
Sample stratification mid-stratum	120	5.8%		105	5.3%	
Sample stratification high-stratum	120	11.9%		105	12.9%	
Mother mid-range age at pregnancy	120	56.6%		105	60.0%	
Mother older age at pregnancy	120	27.1%		105	26.5%	
Mother education >18 years	120	58.0%		105	57.7%	
Mother smoked in pregnancy	120	15.0%		105	21.2%	
Living with partner at conception	120	79.1%		105	75.8%	
Most deprived quintile	120	44.3%		105	39.5%	
Mother anxiety 32 weeks pregnancy	120	31.58	8.44	105	32.15	9.20
Mother depression 32 weeks pregnancy	120	7.12	3.92	105	7.30	4.30
Mother depression factor score from child age 2.5 years to 5 years	120	0.08	1.01	105	-0.10	0.99

Note: Mother mid-range age = 21-30 years, mother older age = >30 years.

Table 2. SEM estimates of covariate effects on the CU traits factor and on the internalizing factor for a weighted sample of 225 children

Model	Covariate	Effects on CU traits		Effects on internalizing behaviours	
		Standardized coefficient	p	Standardized coefficient	p
1	No confounders				
	Male sex	-0.778	.001	-0.553	.013
	Maternal cortisol	-0.602	<.001	-0.288	.004
	Sex X maternal cortisol	1.01	<.001	0.660	.006
2	Confounders (not shown)	-0.657	.001	-0.273	.082
	Male sex				
	Maternal cortisol	-0.447	<.001	-0.196	.065
	Male sex X maternal cortisol	0.858	<.001	0.411	.068
3	Confounders (not shown)	-0.569	<.001	-0.242	.242
	Male sex				
	Maternal cortisol	-0.417	<.001	-0.150	.129
	Male sex X maternal cortisol	0.775	<.001	0.287	.174
	Maternal depression [†]	0.249	.001	0.399	<.001
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4(a,b)	Confounders (not shown)	-0.407	.028	0.094	.616
	Male sex				
	Maternal cortisol	-0.321	.001	0.100	.333
	Male sex X maternal cortisol	0.584	.003	-0.174	.395
	Maternal depression [†]	-0.011	.883	.248	<.001
	Child internalizing factor	0.655	<.001		
	Child CU factor			0.597	<.001

[†]Maternal depression at time of reporting to account for potential reporting bias.