Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Biol Psychiatry. Author manuscript; available in PMC 2020 January 15.

Published in final edited form as:

Biol Psychiatry. 2019 January 15; 85(2): 172–181. doi:10.1016/j.biopsych.2018.06.023.

Maternal Cortisol Concentrations During Pregnancy and Sex Specific Associations with Neonatal Amygdala Connectivity and Emerging Internalizing Behaviors

Alice M. Graham[a], **Jerod M. Rasmussen**[b], **Sonja Entringer**[b,c], **Elizabeth Ben Ward**[d], **Marc D. Rudolph**[e], **John H. Gilmore**[f], **Martin Styner**[f], **Pathik D. Wadhwa**[b], **Damien A. Fair**[a,g], and **Claudia Buss**[b,c]

[a]Department of Behavioral Neuroscience, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd., Portland, OR, 97239, United States;

[b]Development, Health and Disease Research Program, University of California, Irvine, 837 Health Sciences Drive, Irvine, California, 92697, United States;

[c]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health (BIH), Department of Medical Psychology;

[d]Department of Computer Science, University of California, Irvine;

[e]Department of Psychology and Neuroscience, University of North Carolina, 235 E. Cameron Avenue, Chapel Hill, North Carolina, 27599;

[f]Department of Psychiatry, University of North Carolina, 333 South Columbia Street, Chapel Hill, North Carolina, 27514;

[g]Advanced Imaging Research Center, Oregon Health & Science University

Abstract

Background: Maternal cortisol during pregnancy has the potential to influence rapidly developing fetal brain systems that are commonly altered in neurodevelopmental and psychiatric disorders. Research examining maternal cortisol concentrations across pregnancy and offspring neurodevelopment proximal to birth is needed to advance understanding in this area, and lead to insight into the etiology of these disorders.

Methods: Participants were N=70 adult women recruited in early pregnancy, and their infants born after 34-weeks gestation. Maternal cortisol concentrations were assessed serially over four

Financial Disclosures

Dr. Graham reported no biomedical financial interests or potential conflicts of interest.
Dr. Rasmussen reported no biomedical financial interests or potential conflicts of interest.
Dr. Entringer reported no biomedical financial interests or potential conflicts of interest.
Dr. Ward reported no biomedical financial interests or potential conflicts of interest.
Mr. Rudolph reported no biomedical financial interests or potential conflicts of interest.
Dr. Gilmore reported no biomedical financial interests or potential conflicts of interest.
Dr. Styner reported no biomedical financial interests or potential conflicts of interest.
Dr. Wadhwa reported no biomedical financial interests or potential conflicts of interest.
Dr. Fair reported no biomedical financial interests or potential conflicts of interest.
Dr. Buss reported no biomedical financial interests or potential conflicts of interest.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

days in early, mid, and late gestation. Resting state functional connectivity MRI of the neonatal amygdala was examined. Mothers reported on children's internalizing behavior problems at 24-months-of-age.

Results: Maternal cortisol concentrations during pregnancy were significantly associated with neonatal amygdala connectivity in a sex specific manner. Elevated maternal cortisol was associated with stronger amygdala connectivity to brain regions involved in sensory processing and integration, as well as the default mode network in females, and with weaker connectivity to these brain regions in males. Elevated maternal cortisol was associated with higher internalizing symptoms in females only, and this association was mediated by stronger neonatal amygdala connectivity.

Conclusions: Normative variation in maternal cortisol during pregnancy is associated with the coordinated functioning of the amygdala soon after birth in a sex specific manner. The identified pathway from maternal cortisol to higher internalizing symptoms in females via alterations in neonatal amygdala connectivity may be relevant for the etiology of sex differences in internalizing psychiatric disorders, which are more prevalent in females.

Keywords

cortisol; pregnancy; infancy; amygdala; internalizing; resting state functional connectivity MRI

Introduction

Despite advancements in characterizing alterations in brain functioning underlying neurodevelopmental and psychiatric disorders, the disease burden and challenges of successful treatment remains high(1–4), highlighting the need to increase understanding of the etiology of these neural alterations and opportunities for prevention. Maternal glucocorticoids (GCs; cortisol in humans) during pregnancy are of particular interest in this regard due to their: (1) role as a common biomarker across multiple adverse prenatal conditions known to increase risk for offspring psychiatric disorders; (2) capacity to influence fetal GC exposure through multiple mechanisms; and (3) obligatory role in fetal brain development.

Cortisol, the end product of the hypothalamic-pituitary-adrenal (HPA) axis, plays a key role in the body's response to psychological and physiological stress and maintenance of homeostasis(5). It is thus not surprising that cortisol production and bioavailability is altered in association with a range of adverse conditions (e.g. history of childhood maltreatment(6–8), heightened psychosocial stress(9–12), and psychopathology(13, 14)), which may precede or occur during pregnancy, and are known to program offspring risk for neurodevelopmental and psychiatric disorders(15, 16). Maternal cortisol levels during pregnancy can influence fetal cortisol exposure both through stimulation of placental corticotropin-releasing hormone (CRH), which in turn stimulates fetal cortisol production(17–19), and through passage of maternal cortisol through the placenta. The placental enzyme 11β-hydroxysteroid dehydrogenase 2 (11β -HSD2) only forms a partial barrier to the passage of active cortisol(20), and can be down regulated in the context of adverse prenatal conditions(21, 22). Accordingly, positive correlations between maternal cortisol and cortisol in the fetal

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

compartment have been reported(23–25). The obligatory role of cortisol in the developing fetal brain and other organ systems is highlighted by the expression of GC receptors in most fetal tissue, and the necessity of these receptors for survival of the fetus(26). In the fetal brain, GCs play a role in multiple aspects of development, including neurogenesis, gliogenesis, synaptogenesis, and growth of axons and dendrites(27, 28).

The amygdala, which develops at an early embryonic stage(29) and contains a high concentration of GC receptors(30, 31), can alter its developmental trajectory in dependence of varying GC concentrations. Animal models provide experimental evidence for effects of heightened maternal GCs during pregnancy on amygdala development(32, 33), and associated elevations in stress reactivity and anxiety-like behaviors in offspring(32, 34–36). In humans, altered functioning of the amygdala is implicated in those behavioral phenotypes associated with elevated maternal cortisol concentrations during pregnancy or synthetic GC exposure, including heightened stress reactivity and negative emotionality(37–39), and higher levels of internalizing symptoms(40–42).

Animal models and research in humans have identified sex differences in the effects of elevated maternal GCs during pregnancy on offspring development(43, 44). Several studies in humans suggest that heightened negative emotionality and development of internalizing symptoms is more common in female offspring(40, 41, 45, 46). However, only one study has explicitly examined the role of the amygdala in the link between elevated maternal cortisol and risk for internalizing symptoms(40). This study identified an association between heightened maternal cortisol during pregnancy and larger right amygdala volume in school-aged girls, and a pathway from higher maternal cortisol to greater internalizing in girls via this altered amygdala phenotype(40).

The scientific literature to date thus suggests a potentially important role for maternal cortisol during pregnancy in influencing offspring brain development with implications for subsequent internalizing behaviors, which may be more pronounced in females. The current study advances this line of work in several ways. First, prior studies in humans have examined maternal cortisol during pregnancy at a single time point during the day in relation to offspring brain development(40, 41, 47). In contrast, we employ ambulatory cortisol assessment over multiple days in early, mid, and late gestation, allowing for reliable and comprehensive estimation of overall maternal cortisol output during pregnancy. Second, while prior studies have examined brain development in school-aged children(40, 41, 47), the current study focuses on the neonatal brain, increasing capacity to differentiate effects of maternal cortisol concentrations during pregnancy from exposure to postnatal environmental stressors, as well as the influence of heightened offspring stress reactivity and internalizing over time.

Third, this study employs resting state functional connectivity MRI (rs-fcMRI) to examine the coordinated functioning of the neonatal amygdala with other brain regions. Rs-fcMRI reveals information about the functional architecture of the brain beginning in early infancy, and is sensitive enough to capture individual differences which relate to emerging behavioral phenotypes relevant for psychiatric disorders(48–51). We therefore examine maternal cortisol concentrations during pregnancy in relation to neonatal amygdala functional

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

connectivity. Based on prior research examining other stress related processes during pregnancy(49, 52) and stress exposure later in life(53–55), we expect to see altered amygdala functional connectivity to multiple brain regions involved in different sensory, emotional and cognitive functions. We will test associations between the identified patterns of neonatal amygdala connectivity and subsequent child internalizing behavior. We hypothesize that elevated maternal cortisol concentrations during pregnancy will be associated with neonatal amygdala connectivity and subsequent internalizing behaviors in a sex specific manner, such that they confer increased risk for internalizing behaviors in females.

Methods

Participants

Mothers and children in this study (N=70 with neonatal rs-fcMRI data and N=45 with behavioral data at 24-months-of-age) were part of an ongoing prospective, longitudinal study, conducted at the University of California, Irvine, for which mothers were recruited in early pregnancy. Exclusionary criteria were as follows: maternal age < 18 years; maternal use of psychotropic medications or systemic corticosteroids during pregnancy; infant birth before 34 weeks gestation; and infant congenital, genetic, or neurologic disorder. Demographic characteristics are presented in Table 1. A very small portion of mothers reported a mental health diagnosis at study entry (N=2). Behavioral follow-up data were obtained when children were 24-months-of-age (M=24.00 months, SD=.866). There were no significant differences in maternal cortisol concentrations or demographics for those lost to follow-up versus the sample with behavioral follow-up data. See Table S1 for a comparison of demographics for the full sample versus the follow-up sample. All procedures were approved by the Institutional Review Board at the University of California, Irvine, and written informed consent was obtained from all mothers.

Maternal Cortisol Concentrations

As described in our previous work(56) and in the Supplemental Materials, pregnant women collected saliva samples 5 times over the day for 4 consecutive days in early (T1), mid (T2), and late pregnancy (T3; see Table 2), resulting in 60 samples per woman across pregnancy (Figure S1). For each day, total cortisol output was estimated using area under the curve (AUC) with respect to ground. AUC values across days within each time point were significantly correlated (T1, r=.474 - .838, p< .001; T2, r=.630 - .800, p< .001; T3, r=.370 - .700, p< .001), and were averaged to create a reliable indicator of cortisol output at each stage of pregnancy. These average AUC values were base 2 logarithm transformed to bring outliers closer to the mean and normalize the distributions. These values were also highly correlated (T1–T2 r=.664, p< .001; T1–T3 r = .649, p< .001; T2–T3 r= .743, p< .001), and were therefore averaged to create a composite representing overall cortisol output during pregnancy, which is the focus of analyses.

MRI and fMRI Data Acquisition and Processing

Data acquisition.—Neuroimaging data was collected at approximately 4 weeks-of-age (M=3.65, SD=1.72) during natural sleep on a TIM Trio, Siemens Medical System 3.0T

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

scanner. High resolution T2- (TR=3200ms, echo time=255ms, resolution=1×1×1mm, 4:18 mins) and T1-weighted scans (MP-RAGE TR=2400ms, inversion time=1200ms, echo time=3.16ms, flip angle=8°, resolution=1×1×1mm, 6:18 mins) were collected. Functional images for resting state functional connectivity MRI (rs-fcMRI) were obtained using a gradient-echo, echoplanar imaging (EPI) sequence sensitive to blood oxygen level-dependent contrast (TR=2000ms; TE=30ms; FOV=220×220×160mm; 195 TRs; 32 interleaved-ascending axial slices; 1mm gap; resolution=3.4×3.4×4mm; flip angle=77°).

MRI and fMRI data preprocessing.—Processing followed established procedures for neuroimaging with neonates as described in our previous work(48, 49) and in the Supplemental Materials.

rs-fcMRI preprocessing.—Additional preprocessing steps for rs-fcMRI were implemented to account for signal stemming from non-neuronal processes (57–59) as described in the Supplemental Materials. Volume censoring was employed to reduce effects of motion determined by framewise displacement (FD)(60) of .3mm. Remaining mean FD was subsequently examined as a potential confound. As in our prior work(48, 49), seed regions for rs-fcMRI analysis were individually segmented amygdala (see Supplemental Materials).

Potential Confounds Relevant for Maternal Cortisol and Neonatal Brain Outcomes

Potential confounds relevant to maternal cortisol concentrations and infant brain development were examined. These included maternal pre-pregnancy body mass index (BMI), maternal cigarette smoking during pregnancy, obstetric (OB) risk, annual household income, and maternal systemic inflammation during pregnancy (as indexed by interleukin-6 [IL-6]). See Supplemental Materials.

Internalizing Behavior at 24-months-of-age

Mothers reported on children's internalizing behavior problems at 24-months-of-age on the Internalizing Behavior scale (α=.966) of the Child Behavior Checklist/1.5–5 (CBCL;(61)).

Maternal Postnatal Depression

The 20-item Center for Epidemiological Studies of Depression Scale (CESD;(62)) was used to assess maternal depression symptoms repeatedly over the first 2-years of life, and a composite score was used in analyses (see Supplemental Materials).

Analyses

The interaction between mean maternal cortisol AUC and fetal sex was regressed on whole-brain amygdala voxelwise connectivity while adjusting for the main effects of mean maternal cortisol and infant sex, as well as infant gestational age at birth (GA) and age at scan. This whole-brain approach was deemed appropriate given our expectation that amygdala connectivity with multiple brain regions would be altered, and the lack of prior research examining amygdala connectivity in relation to maternal cortisol during pregnancy. Left and right amygdala connectivity were examined separately due to evidence for lateralized effects of prenatal influences(52) and asymmetry in relation to psychiatric

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

outcomes(63). Multiple comparisons correction for $p < .05$ voxel clusters required a threshold of 35 contiguous voxels with a Z value > 2.50 based on Monte Carlo simulation(89). Connections identified in the whole brain analyses were extracted (see Supplemental Methods for details). To probe the interaction, correlations between maternal cortisol and these connections were examined separately for females and males. Next, we tested for potential confounding influences on each extracted connection.

We then tested associations between the strongest results from the whole-brain analyses (based on Z value) and child internalizing behaviors at 24-months-of-age. A covariate for maternal postnatal depression was included to account for variation in the postnatal environment, and potential bias in maternal report on child internalizing. Next, we examined the direct association between maternal cortisol and child internalizing behaviors, considering infant sex as a moderator, and postnatal depression as a covariate. Finally, we planned to examine neonatal amygdala connectivity as a mediator of any identified association between maternal cortisol during pregnancy and child internalizing using a structural equation modeling framework Mplus, Version 7(64).

Results

The Association between Maternal Cortisol During Pregnancy and Neonatal Amygdala Connectivity is Moderated by Infant Sex

The interaction between maternal cortisol during pregnancy and infant sex was significantly associated with neonatal amygdala connectivity. For the right amygdala, the maternal cortisol – infant sex interaction was significantly associated with connectivity to the following regions: left supramarginal gyrus (SMG) and superior temporal gyrus (STG), and right inferior temporal gyrus (ITG; extending into fusiform gyrus) and dorsolateral prefrontal cortex (DLPFC). Results for the left amygdala were consistent with regard to the left SMG, right ITG, and a left STG region (although more rostral and less extensive). However, findings for the left amygdala also included the right and left precuneus and a more ventral and rostral fusiform gyrus region (Table 3 and Figure 1).

Probing the Interaction between Maternal Cortisol and Infant Sex Reveals Distinct Effects for Females versus Males

We extracted all significant connections identified in the whole-brain analysis (see Supplemental Materials) and examined the Pearson correlations between maternal cortisol and these connections separately for males versus females. Overall, for females, higher maternal cortisol was associated with stronger right and left amygdala connectivity to cortical brain regions. In contrast, for males, higher maternal cortisol was associated with weaker amygdala connectivity to these regions. Right amygdala-DLPFC and left amygdala-fusiform gyrus connectivity were the two exceptions, such that higher maternal cortisol was associated with weaker connectivity in females, and stronger connectivity in males (Table 3). These results suggest a cross-over interaction, indicating that sex differences were not characterized by a stronger association for one sex compared to the other, but rather the associations between maternal cortisol and neonatal amygdala connectivity were opposite for females versus males.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Potentially Confounding Factors Do Not Impact the Associations between Maternal Cortisol and Neonatal Amygdala Connectivity

The interaction between maternal cortisol during pregnancy and infant sex remained significantly associated with all of the identified connections ($p < .05$) after adjusting for all of the potential confounds, as well as remaining FD (micro-movements during functional data acquisition remaining after frame removal).

Neonatal Amygdala Phenotypes Associated with Maternal Cortisol during Pregnancy are Relevant for Child Internalizing Behavior

The strongest finding for the right amygdala (based on Z-value), amygdala-SMG connectivity, was positively associated with child internalizing behavior at 24-months-of-age (β= .336, p=.017). Infant sex was not a significant moderator (β= .058, p=.737). Of the covariates in the model, higher maternal postnatal depression was significantly associated with greater child internalizing (β=.408, p=.004).

The strongest finding for the left amygdala, left amygdala-ITG connectivity, was positively associated with child internalizing behavior only at the trend level (β=.252, p=.094). Infant sex was again not a significant moderator (β= −.190, p=.301), and the covariate for maternal depression was associated with child internalizing (β= .349, p=.018). Thus, patterns of neonatal amygdala connectivity associated with maternal cortisol during pregnancy are relevant for later emerging child internalizing symptoms. These associations are not modulated by infant sex, indicating that the moderation effect is specific to the association between maternal cortisol during pregnancy and the neonatal brain phenotypes.

The Association between Maternal Cortisol During Pregnancy and Child Internalizing Behavior is Moderated by Infant Sex

The interaction between maternal cortisol and infant sex was significantly associated with child internalizing (β= 1.527, p=.026). The covariate for maternal postnatal depression was also significantly associated with internalizing (β= .390, p=.010). Probing the interaction revealed that maternal cortisol was significantly associated with higher internalizing behaviors for females (β=.533, p=.013), but not for males (β= −.175, p=.358).

Neonatal Amygdala-SMG Connectivity Mediates the Association between Maternal Cortisol during Pregnancy and Subsequent Internalizing Behavior in a Sex Dependent Manner

Based on the significant effect of maternal cortisol on child internalizing, and the significant pathways from maternal cortisol to neonatal amygdala connectivity, and from amygdala-SMG connectivity to internalizing, we tested for mediation. We used a moderated mediation model to account for the moderating effect of infant sex. For females, higher maternal cortisol was associated with higher child internalizing via stronger right-amygdala-SMG connectivity (indirect effect= 3.14 ; 95% CI: .198, 10.2; based on 5,000 bootstrap samples). Interestingly, the indirect path was also significant for males, such that higher maternal cortisol was associated with lower child internalizing via weaker right amygdala-SMG connectivity (indirect effect= −3.61; 95% CI: −10.3, −.645; based on 5,000 bootstrap samples). Thus for females, the direct effect of maternal cortisol during pregnancy on child internalizing is mediated by higher amygdala-SMG connectivity. In males, there was no

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

direct association between maternal cortisol and subsequent internalizing, but the significant mediation result suggests that heightened maternal cortisol during pregnancy is associated with lower internalizing behavior via this specific pathway involving amygdala-SMG connectivity. The lack of a direct effect for males indicates that other pathways exist through which heightened maternal cortisol has the opposite effect on internalizing behavior in males.

Discussion

Summary of Findings

Although extensively studied in animal models, the implications of variability in maternal cortisol concentrations during pregnancy for offspring brain and behavioral development in humans are not well understood. The findings of the current study indicate that normative variation in maternal cortisol concentrations during pregnancy are associated with alterations in neonatal amygdala functional connectivity to multiple cortical brain regions involved in sensory processing and integration, the default mode network (DMN), and emotion regulation. These associations differed by offspring sex, such that elevated maternal cortisol was associated with stronger neonatal amygdala connectivity in females, and weaker connectivity in males. The amygdala connections most strongly associated with maternal cortisol in turn predicted internalizing behavior when children were two-years-of-age, after accounting for maternal postnatal depressive symptoms. A significant direct effect of maternal cortisol during pregnancy on child internalizing was identified in females only. Stronger neonatal amygdala connectivity mediated the effect of elevated maternal cortisol on higher internalizing in females. We consider the potential implications of these findings for the etiology of sex differences in internalizing psychiatric disorders, which are more prevalent in females(65–68).

Potential Mechanisms Relevant to Observed Sex Differences

Research to date suggests several potential mechanisms, at the level of the placenta and fetal brain, through which maternal cortisol concentrations during pregnancy may exert sex specific effects on fetal neurodevelopment, and particularly brain regions such as the amygdala, with high potential to be influenced by GCs. The placenta is an organ of fetal origin, and therefore has the same X and Y chromosome composition as the fetus(69). Sex differences in placental gene expression and regulation, including of the placental enzyme 11β-HSD2(70, 71) and placental GC receptor isoforms(72–75), affect both the passage of active maternal GCs to the fetal compartment and the extent to which maternal GCs stimulate fetal GC production. These mechanisms lead to sex differences in fetal GC exposure and associated phenotypic alterations in the context of elevated maternal GCs(44, 70–77). Sex differences in the timing and pattern of GC receptor expression in the developing fetal brain have also been observed, and likely contribute to differences in how GC exposure shapes ongoing neural development(78–80).

Brain Regions and Networks Involved and Relation to Internalizing Symptoms

Results of the present study reveal a pattern of increased amygdala connectivity for females in association with elevated maternal cortisol concentrations. In adults, stronger amygdala

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

connectivity has been observed following stress exposure(53–55), and in association with higher subclinical and pathological internalizing symptoms(81–83). This finding is also in line with the limited prior research examining infant amygdala functional connectivity in relation to other indicators of prenatal adversity (heightened maternal systemic inflammation(49) and depressive symptoms(52)), suggesting that various forms of psychological stress and biological stress mediators during pregnancy may result in a neural phenotype of increased amygdala integration into early emerging functional brain architecture.

The findings for females specifically indicate stronger amygdala connectivity to brain regions involved in sensory processing and integration (SMG and STG), as well as the DMN (precuneus and ITG). Increased coordinated functioning of the amygdala with sensory processing and integration regions may be indicative of heightened sensitivity to sensory information, and has been observed in patients with subclinical and clinical anxiety(81, 84–87), and disorders involving chronic pain(88, 89). Heightened functional connectivity of the amygdala with regions of the DMN has been observed in response to environmental stress (90) (91) and in association with internalizing disorders(92, 93). Interestingly, heightened maternal cortisol was also associated with females showing weaker functional connectivity of amygdala to DLPFC, a region involved in effectively regulating negative emotions(94). Taken together, the findings suggest a neural phenotype potentially indicative of increased sensitivity and vulnerability to experiencing heightened negative emotionality with decreased capacity for emotion regulation. This interpretation is supported by the association of stronger neonatal amygdala connectivity to the SMG, an important region for multimodal sensory integration(95–98), with higher subsequent internalizing behavior.

Males displayed the opposite pattern, such that amygdala connectivity to these sensory processing and integration regions, as well as the DMN, was weaker, and amygdala-DLPFC connectivity was stronger in association with heightened maternal cortisol. This neural phenotype could be indicative of decreased sensitivity to sensory stimuli and increased capacity for emotion regulation. Interestingly, prior research has indicated that alterations in the coordinated functioning of the amygdala associated with exposure to postnatal environmental stress may confer resilience to internalizing symptoms(99, 100). This may be the case for males in the context of exposure to heightened maternal cortisol during pregnancy as weaker neonatal amygdala-SMG connectivity was associated with lower internalizing symptoms at 2-years-of-age.

Stronger neonatal amygdala connectivity mediated the association between elevated maternal cortisol during pregnancy and higher internalizing symptoms in females. For males, there was no direct association between maternal cortisol and internalizing symptoms. However, the significant indirect effect of heightened maternal cortisol on internalizing behavior via altered amygdala-SMG connectivity suggests one potential pathway through which heightened cortisol could lead to lower internalizing behaviors in males. This finding is in line with a recent large study, which identified an association between heightened maternal cortisol during pregnancy and lower negative emotionality in male infants(45).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

These findings may suggest one early point of divergence in terms of later risk for internalizing psychiatric disorders, which are known to be more prevalent in females(65–68) beginning during puberty(101). Future research will be needed to examine whether these early sex specific alterations in brain connectivity and behavior increase sensitivity to the hormonal and social changes of puberty, or even relate to shifts in pubertal timing, which are in turn associated with heightened internalizing in females(102). It is important to note that while females are at greater risk of internalizing disorders, males are at greater risk for other psychiatric diagnoses, including autism, substance abuse, and externalizing disorders(65, 67, 103). Ongoing research following brain and behavioral development across infancy and childhood will be needed to delineate such pathways of risk and resilience for males and females.

Limitations and Alternative Explanations for Findings

Several limitations of this study should be considered. First, cortisol does not act in isolation to influence the developing fetus. The endocrine system interacts with others, including the immune system, with potential to influence fetal neurodevelopment(49). While results of the current study remained consistent after adjusting for maternal systemic inflammation, we did not have sufficient statistical power to examine the interactive and cumulative influence of these systems, which will be a critical topic for future studies. With regard to genetic contributions to internalizing behavior, maternal stress during pregnancy has been found to be similarly associated with elevated child anxiety when mothers carry genetically related (via in vitro fertilization) or unrelated children (via in vitro fertilization with egg donation), suggesting a large environmental influence(104). Our analyses also adjust for maternal postnatal depression over a two-year period, making it unlikely that shared genetic vulnerability to internalizing symptoms explains these findings.

With regard to the neuroimaging results, although we hypothesized that maternal cortisol concentrations would be associated with altered neonatal amygdala connectivity to multiple brain regions spanning sensory, emotional, and cognitive functions, we did not set forth hypotheses about specific regions, and thus our interpretations regarding these connections are post-hoc. Similarly, the lateralized findings, including left SMG and STG, and right ITG and DLPFC, are consistent with prior research indicating asymmetry in effects of prenatal stress exposure on neurodevelopment(87), but were not specifically hypothesized. It should also be noted that neuroimaging with infants, by necessity, occurred during natural sleep. Ongoing investigation into the differences in coordinated brain functioning during infant sleep and wake cycles will likely aide in interpretation of findings moving forward. Lastly, the measure of child internalizing symptoms was based on maternal report, and could be improved by the addition of a diagnostic interview and observational assessment.

Conclusion

The findings of the present study provide support for the sensitivity of developing fetal limbic brain circuitry to variation in maternal cortisol during pregnancy in a normative sample of pregnant women. This work builds on prior research(40, 41, 45), and provides novel insight into a potential pathway through which prenatal conditions may lead to increased risk for internalizing symptomatology in females. More broadly, these findings

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

advance understanding of one key aspect of maternal stress biology during pregnancy in relation to offspring brain and behavioral development. This work can provide a foundation for subsequent investigations, including examination of the multitude of factors which may contribute to elevated maternal cortisol levels during gestation, and the longer term sequelae of the observed alterations in offspring amygdala connectivity and emerging internalizing behaviors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Support for this work was provided by NICHD R01 HD060628 (Wadhwa; EMA Assessment of Biobehavioral Processes in Human Pregnancy), NIMH R01 MH091351 (Buss &Wadhwa; Fetal Programming of the Newborn and Infant Human Brain), Supplement to R01 MH091351 (Buss & Fair; Fetal Programming of Brain Functional Connectivity in Neonates and Infants), NIMH K99 MH111805 (Graham; A targeted approach to examine the influence of maternal psychological stress on newborn brain outcomes), Gates Foundation Grand Challenges Explorations (Fair & Nardos; Early Markers to Predict Cognition and Brain Development), and R00 MH091238 (Fair), R01 MH096773 (Fair).

References

1. National Institute of Mental Health (2016): Statistics. . Retrieved from http://www.nimh.nih.gov/health/statistics/cost/index.shtml.
2. Levit K, Kassed C, Coffey R, Mark T, McKusick D, King E, et al. (2008): Projections of National Expenditures for Mental Health Services and Substance Abuse Treatment, 2004-–2014. Rockville, MD.
3. Huhn M, Tardy M, Spineli LM, Kissling W, Förstl H, Pitschel-Walz G, et al. (2014): Efficacy of pharmacotherapy and psychotherapy for adult psychiatric disorders: a systematic overview of meta-analyses. JAMA Psychiatry. 71: 706–15. [PubMed: 24789675]
4. Lynch D, Laws KR, McKenna PJ (2010): Cognitive behavioural therapy for major psychiatric disorder: does it really work? A meta-analytical review of well-controlled trials. Psychol Med. 40: 9–24. [PubMed: 19476688]
5. Sapolsky RM, Romero L, Munck AU (2000): How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev. 21: 55–89. [PubMed: 10696570]
6. Schreier HMC, Bosquet Enlow M, Ritz T, Coull BA, Gennings C, Wright RO, Wright RJ (2016): Lifetime exposure to traumatic and other stressful life events and hair cortisol in a multi-racial/ethnic sample of pregnant women. Stress. 19: 45–52. [PubMed: 26551892]
7. Schreier HMC, Enlow MB, Ritz T, Gennings C, Wright RJ (2015): Childhood abuse is associated with increased hair cortisol levels among urban pregnant women. J Epidemiol Community Health. 69: 1169–1174. [PubMed: 26219886]
8. Bublitz MH, Stroud LR (2012): Childhood sexual abuse is associated with cortisol awakening response over pregnancy: Preliminary findings. Psychone. 37: 1425–1430.
9. Simon CD, Adam EK, Holl JL, Wolfe KA, Grobman WA, Borders AEB (2016): Prenatal Stress and the Cortisol Awakening Response in African-American and Caucasian Women in the Third Trimester of Pregnancy. Matern Child Health J. 20: 2142–2149. [PubMed: 27392704]
10. Entringer S, Buss C, Andersen J, Chicz-DeMet A, Wadhwa PD (2011): Ecological momentary assessment of maternal cortisol profiles over a multiple-day period predicts the length of human gestation. Psychosom Med. 73: 469–474. [PubMed: 21700714]
11. Giesbrecht GF, Campbell T, Letourneau N, Kooistra L, Kaplan B (2012): Psychological distress and salivary cortisol covary within persons during pregnancy. Psychoneuroendocrinology. 37: 270–9. [PubMed: 21752548]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

12. Kane HS, Schetter CD, Glynn LM, Hobel CJ, Sandman CA (2014): Pregnancy Anxiety and Prenatal Cortisol Trajectories. Biol Psychol. 100: 13–19. [PubMed: 24769094]
13. Caparros-Gonzalez RA, Romero-Gonzalez B, Strivens-Vilchez H, Gonzalez-Perez R, Martinez-Augustin O, Peralta-Ramirez MI (2017): Hair cortisol levels, psychological stress and psychopathological symptoms as predictors of postpartum depression. PLoS One. 12: 1–17.
14. O'Connor TG, Tang W, Gilchrist M a, Moynihan J a, Pressman EK, Blackmore ER (2014): Diurnal cortisol patterns and psychiatric symptoms in pregnancy: short-term longitudinal study. Biol Psychol. 96: 35–41. [PubMed: 24239618]
15. Entringer S, Buss C, Wadhwa PD (2011): Prenatal stress and developmental programming of human health and disease risk: concepts and integration of empirical findings. Curr Opin Endocrinol Diabetes Obes. 17: 1–18.
16. Buss C, Entringer S, Moog NK, Toepfer P, Fair DA, Simhan HN, et al. (2017): Intergenerational Transmission of Maternal Childhood Maltreatment Exposure: Implications for Fetal Brain Development. J Am Acad Child Adolesc Psychiatry. 56: 373–382. [PubMed: 28433086]
17. Sandman C a, Glynn L, Schetter CD, Wadhwa P, Garite T, Chicz-DeMet A, Hobel C (2006): Elevated maternal cortisol early in pregnancy predicts third trimester levels of placental corticotropin releasing hormone (CRH): priming the placental clock. Peptides. 27: 1457–63. [PubMed: 16309788]
18. Rehman KS, Sirianni R, Parker CR, Rainey WE, Carr BR (2007): The regulation of adrenocorticotrophic hormone receptor by corticotropin-releasing hormone in human fetal adrenal definitive/transitional zone cells. Reprod Sci. 14: 578–587. [PubMed: 17959886]
19. Cheng YH, Nicholson RC, King B, Chan EC, Fitter JT, Smith R (2000): Glucocorticoid stimulation of corticotropin-releasing hormone gene expression requires a cyclic adenosine 3', 5'-monophosphate regulatory element in human primary placental cytotrophoblast cells. J Clin Endocrinol Metab. 85: 1937. [PubMed: 10843178]
20. Benediktsson R, Calder AA, Edwards CRW, Seckl JR (1997): Placental 11β-hydroxysteroid dehydrogenase: A key regulator of fetal glucocorticoid exposure. Clin Endocrinol (Oxf). 46.
21. Reynolds RM (2013): Glucocorticoid excess and the developmental origins of disease: Two decades of testing the hypothesis - 2012 Curt Richter Award Winner. Psychoneuroendocrinology. 38: 1–11. [PubMed: 22998948]
22. O'Donnell KJ, Bugge Jensen A, Freeman L, Khalife N, O'Connor TG, Glover V (2012): Maternal prenatal anxiety and downregulation of placental 11β-HSD2. Psychoneuroendocrinology. 37: 818–826. [PubMed: 22001010]
23. Glover V, Bergman K, Sarkar P, O'Connor TG (2009): Association between maternal and amniotic fluid cortisol is moderated by maternal anxiety. Psychoneuroendocrinology. 34: 430–5. [PubMed: 19019559]
24. Baibazarova E, Van De Beek C, Cohen-Kettenis PT, Buitelaar J, Shelton KH, Van Goozen SHM (2013): Influence of prenatal maternal stress, maternal plasma cortisol and cortisol in the amniotic fluid on birth outcomes and child temperament at 3 months. Psychoneuroendocrinology. 38: 907–915. [PubMed: 23046825]
25. Gitau R, Cameron A, Fisk NM, Glover V (1998): Fetal exposure to maternal cortisol. Lancet (London, England). 352: 707–8.
26. Cole TJ, Blendy J a, Monaghan a P (1995): Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell …. Genes Dev. 1608–1621. [PubMed: 7628695]
27. Moisiadis VG, Matthews SG (2014): Glucocorticoids and fetal programming part 2: Mechanisms. Nat Rev Endocrinol. 10: 403–411. [PubMed: 24863383]
28. Matthews SG (2000): Antenatal Glucocorticoids and Programming of the Developing CNS. Pediatr Res. 47: 291–300. [PubMed: 10709726]
29. Humphrey T (1968): The development of the human amygdala during early embryonic life,. J Comp Neurol. 132: 135–165. [PubMed: 5732427]
30. Reul J, de Kloet ER (1985): Two Receptor Systems for Corticosterone in Rat Brain: Microdistribution and Differential Occupation. Endocrinology. 117: 2505–2511. [PubMed: 2998738]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

31. Wang Q, Verweij EWE, Krugers HJ, Joels M, Swaab DF, Lucassen PJ (2014): Distribution of the glucocorticoid receptor in the human amygdala; changes in mood disorder patients. Brain Struct Funct. 219: 1615–1626. [PubMed: 23748930]
32. Welberg LA, Seckl JR, Holmes MC (2000): Inhibition of 11beta-hydroxysteroid dehydrogenase, the foetoplacental barrier to maternal glucocorticoids, permanently programs amygdala GR mRNA expression and anxiety-like behaviour in the offspring. Eur J Neurosci. 12: 1047–1054. [PubMed: 10762336]
33. Salm AK, Pavelko M, Krouse EM, Webster W, Kraszpulski M, Birkle DL (2004): Lateral amygdaloid nucleus expansion in adult rats is associated with exposure to prenatal stress. Dev Brain Res. 148: 159–167. [PubMed: 14766193]
34. Shoener JA, Baig R, Page KC, Jennifer A, Pre- KCP (2006): Prenatal exposure to dexamethasone alters hippocampal drive on hypothalamic-pituitary-adrenal axis activity in adult male rats. Am J Physiol Regul Integr Comp Physiol. 290: R1366–R1373. [PubMed: 16397092]
35. Levitt N, Lindsay R, Holmes M, Seckl J (1996): Dexamethasone in the Last Week of Pregnancy Attenuates Hippocampal Glucocorticoid Receptor Gene Expression and Elevates Blood Pressure in the Adult Offspring in the Rat. Neuroendocrinology. 64: 412–418. [PubMed: 8990073]
36. Barbazanges A, Piazza P-V, Le Moal M, Maccari S (1996): Maternal Glucocorticoid Secretion Mediates Long-Term Effects of Prenatal Stress. J Neurosci. 16: 3943–3949. [PubMed: 8656288]
37. Davis EP, Glynn LM, Schetter CD, Hobel C, Chicz-Demet A, Sandman C a (2007): Prenatal exposure to maternal depression and cortisol influences infant temperament. J Am Acad Child Adolesc Psychiatry. 46: 737–46. [PubMed: 17513986]
38. Davis EP, Waffarn F, Sandman CA (2011): Prenatal treatment with glucocorticoids sensitizes the HPA axis response to stress among full-term infants. Dev Psychobiol. 53: 175–83. [PubMed: 21298632]
39. De Weerth C, Van Hees Y, Buitelaar JK (2003): Prenatal maternal cortisol levels and infant behavior during the first 5 months. Early Hum Dev. 74: 139–151. [PubMed: 14580753]
40. Buss C, Davis EP, Shahbaba B, Pruessner JC, Head K, Sandman CA (2012): Maternal cortisol over the course of pregnancy and subsequent child amygdala and hippocampus volumes and affective problems. Proc Natl Acad Sci. 109: E1312–E1319. [PubMed: 22529357]
41. Kim D-J, Davis EP, Sandman CA, Sporns O, O'Donnell BF, Buss C, Hetrick WP (2016): Prenatal Maternal Cortisol Has Sex-Specific Associations with Child Brain Network Properties. Cereb Cortex. 5230–5241.
42. Davis EP, Sandman CA (2012): Prenatal psychobiological predictors of anxiety risk in preadolescent children. Psychoneuroendocrinology. 37: 1224–1233. [PubMed: 22265195]
43. Glover V, Hill J (2012): Sex differences in the programming effects of prenatal stress on psychopathology and stress responses: an evolutionary perspective. Physiol Behav. 106: 736–40. [PubMed: 22353310]
44. Chan JC, Nugent BM, Bale TL (2017): Parental advisory: maternal and paternal stress can impact offspring neurodevelopment. Biol Psychiatry. . doi: 10.1016/j.biopsych.2017.10.005.
45. Braithwaite EC, Pickles A, Sharp H, Glover V, O'Donnell KJ, Tibu F, Hill J (2017): Maternal prenatal cortisol predicts infant negative emotionality in a sex-dependent manner. Physiol Behav. 175: 31–36. [PubMed: 28322912]
46. Van den Bergh BRH, Van Calster B, Smits T, Van Huffel S, Lagae L (2008): 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. 33: 536–45. [PubMed: 17507916]
47. Davis EP, Head K, Buss C, Sandman CA (2017): Prenatal maternal cortisol concentrations predict neurodevelopment in middle childhood. Psychoneuroendocrinology. 75: 56–63. [PubMed: 27771566]
48. Graham AM, Buss C, Rasmussen JM, Rudolph MD, Demeter DV, Gilmore JH, et al. (2016): Implications of newborn amygdala connectivity for fear and cognitive development at 6-months-of-age. Dev Cogn Neurosci. 18: 12–25. [PubMed: 26499255]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

49. Graham AM, Rasmussen JM, Rudolph MD, Heim CM, Gilmore JH, Styner M, et al. (2017): Maternal Systemic Interleukin-6 During Pregnancy Is Associated With Newborn Amygdala Phenotypes and Subsequent Behavior at 2 Years of Age. Biol Psychiatry.
50. Graham AM, Pfeifer JH, Fisher PA, Carpenter S, Fair DA (2015): Early life stress is associated with default system integrity and emotionality during infancy. J Child Psychol Psychiatry. 56: 1212–22. [PubMed: 25809052]
51. Rogers CE, Sylvester CM, Carrie M, Kenley JK, Shimony JS, Barch DM, Smyser CD (2017): Neonatal Amygdala Functional Connectivity at Rest in Healthy and Preterm Infants and Early Internalizing Symptoms. J Am Acad Child Adolesc Psychiatry. 56: 157–166. [PubMed: 28117062]
52. Qiu A, Anh TT, Li Y, Chen H, Rifkin-Graboi A, Broekman BFP, et al. (2015): Prenatal maternal depression alters amygdala functional connectivity in 6-month-old infants. Transl Psychiatry. 5: e508. [PubMed: 25689569]
53. van Marle HJF, Hermans EJ, Qin S, Fernández G (2010): Enhanced resting-state connectivity of amygdala in the immediate aftermath of acute psychological stress. Neuroimage. 53: 348–54. [PubMed: 20621656]
54. Rabinak CA, Angstadt M, Welsh RC, Kenndy AE, Lyubkin M, Martis B, Phan KL (2011): Altered amygdala resting-state functional connectivity in post-traumatic stress disorder. Front Psychiatry. 2: 62. [PubMed: 22102841]
55. Sripada RK, King AP, Garfinkel SN, Wang X, Sripada CS, Welsh RC, Liberzon I (2012): Altered resting-state amygdala functional connectivity in men with posttraumatic stress disorder. J Psychiatry Neurosci. 37: 241–9. [PubMed: 22313617]
56. Entringer S, Buss C, Rasmussen JM, Lindsay K, Gillen DL, Cooper DM, Wadhwa PD (2017): Maternal cortisol during pregnancy and infant adiposity: A prospective investigation. J Clin Endocrinol Metab. 102: 1366–1374. [PubMed: 28009530]
57. Fox MD, Raichle ME (2007): Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neurosci. 8: 700–11. [PubMed: 17704812]
58. Fair DA, Nigg JT, Iyer S, Bathula D, Mills KL, Dosenbach NUF, et al. (2012): Distinct neural signatures detected for ADHD subtypes after controlling for micro-movements in resting state functional connectivity MRI data. Front Syst Neurosci. 6: 80. [PubMed: 23382713]
59. Fair D a, Cohen AL, Power JD, Dosenbach NUF, Church J a, Miezin FM, et al. (2009): Functional brain networks develop from a “local to distributed” organization. PLoS Comput Biol. 5: e1000381. [PubMed: 19412534]
60. Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE (2012): Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. Neuroimage. 59: 2142–2154. [PubMed: 22019881]
61. Achenbach TM, Rescorla L (2001): Manual for the ASEBA School-Age Forms & Profiles. Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families.
62. Radloff LS (1977): Center for Epidemiologic Studies Depression Scale (CESD) Keywords: Background : Developer (s): Copyright : Reliability : Assessment : Scale items : References : Heal (San Fr. 51: 4–6.
63. Okada N, Fukunaga M, Yamashita F, Koshiyama D, Yamamori H, Ohi K, et al. (2016): Abnormal asymmetries in subcortical brain volume in schizophrenia. Mol Psychiatry. 1–7. [PubMed: 26678307]
64. Muthén LK, Muthén BO (2015): Mplus User’s Guide, Seventh. Los Angeles, CA: Muthén & Muthén Retrieved April 29, 2013, from http://www.statmodel.com/download/usersguide/MplusUsersGuidev6.pdf.
65. Bangasser DA, Valentino RJ (2014): Sex differences in stress-related psychiatric disorders: Neurobiological perspectives. Front Neuroendocrinol. 35: 303–319. [PubMed: 24726661]
66. Altemus M, Sarvaiya N, Neill Epperson C (2014): Sex differences in anxiety and depression clinical perspectives. Front Neuroendocrinol. 35: 320–330. [PubMed: 24887405]
67. Kessler RC, Chiu WT, Demler O, Walters EE (2005): Prevalence, Severity, and Comorbidity of 12-Month DSM-IV Disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry. 62: 617–627. [PubMed: 15939839]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

68. Mclean CP, Asnaani A, Litz BT, G HS (2011): Gender differences in anxiety disorders: Prevalence, course of illness, comorbidity and burden of illness. J Psychiatr Res. 45: 1027–1035. [PubMed: 21439576]
69. Rossant J, Cross JC (2001): Placental development: lessons from mouse mutants. Nat Rev Genet. 2: 538–48. [PubMed: 11433360]
70. Cuffe J, O'Sullivan L, Simmons D, Anderson S, Moritz K (2012): Maternal corticosterone exposure in the mouse has sex-specific effects on placental growth and mRNA expression. Endocrinology. 153: 5500–5511. [PubMed: 22919064]
71. Stark M, Wright I, Clifton V (2009): Sex-specific alterations in placental 11 -hydroxysteroid dehydrogenase 2 activity and early postnatal clinical course following antenatal betamethasone. Am J Regul Integr Comp Physiol. 297: R510–R514.
72. Meakin AS, Saif Z, Jones AR, Aviles PFV, Clifton VL (2017): Review: Placental adaptations to the presence of maternal asthma during pregnancy. Placenta. 54: 17–23. [PubMed: 28131319]
73. Saif Z, Hodyl N a., Stark MJ, Fuller PJ, Cole T, Lu N, Clifton VL (2015): Expression of eight glucocorticoid receptor isoforms in the human preterm placenta vary with fetal sex and birthweight. Placenta. 36: 723–730. [PubMed: 25990415]
74. Saif Z, Hodyl NA, Hobbs E, Tuck AR, Butler MS, Osei-Kumah A, Clifton VL (2014): The human placenta expresses multiple glucocorticoid receptor isoforms that are altered by fetal sex, growth restriction and maternal asthma. Placenta. 35: 260–268. [PubMed: 24559727]
75. Scott N, Hodyl N, Osei-Kumah A, Stark M, Smith R, Clifton V (2011): The presence of maternal asthma during pregnancy suppresses the placental pro-inflammatory response to an immune challenge in vitro. Placenta. 32: 454–461. [PubMed: 21453968]
76. Bale TL (2016): The placenta and neurodevelopment: Sex differences in prenatal vulnerability. Dialogues Clin Neurosci. 17: 459–464.
77. Nugent BM, Bale TL (2015): The omniscient placenta: Metabolic and epigenetic regulation of fetal programming. Front Neuroendocrinol. 39: 28–37. [PubMed: 26368654]
78. Owen D, Matthews SG (2003): Glucocorticoids and sex-dependent development of brain glucocorticoid and mineralocorticoid receptors. Endocrinology. 144: 2775–2784. [PubMed: 12810530]
79. Liu LI, Li A, Matthews SG, Li A, Maternal SGM (2001): Maternal glucocorticoid treatment programs HPA regulation in adult offspring : sex-specific effects. Am J Endocrinol Metab. 729–739.
80. Dean F, Matthews SG (1999): Maternal dexamethasone treatment in late gestation alters glucocorticoid and mineralocorticoid receptor mRNA in the fetal guinea pig brain. Brain Res. 846: 253–259. [PubMed: 10556643]
81. Qin S, Young CB, Duan X, Chen T, Supekar K, Menon V (2014): Amygdala subregional structure and intrinsic functional connectivity predicts individual differences in anxiety during early childhood. Biol Psychiatry. 75: 892–900. [PubMed: 24268662]
82. Baur V, Hänggi J, Langer N, Jäncke L (2013): Resting-state functional and structural connectivity within an insula-amygdala route specifically index state and trait anxiety. Biol Psychiatry. 73: 85–92. [PubMed: 22770651]
83. Admon R, Lubin G, Stern O, Rosenberg K, Sela L, Ben-Ami H, Hendler T (2009): Human vulnerability to stress depends on amygdala's predisposition and hippocampal plasticity. Proc Natl Acad Sci U S A. 106: 14120–5. [PubMed: 19666562]
84. Li Y, Qin W, Jiang T, Zhang Y, Yu C (2012): Sex-dependent correlations between the personality dimension of harm avoidance and the resting-state functional connectivity of amygdala subregions. PLoS One. 7: e35925. [PubMed: 22558274]
85. Roy AK, Fudge JL, Kelly AMC, Perry JS a, Daniele T, Carlisi C, et al. (2013): Intrinsic functional connectivity of amygdala-based networks in adolescent generalized anxiety disorder. J Am Acad Child Adolesc Psychiatry. 52: 290–299.e2. [PubMed: 23452685]
86. Greening SG, Mitchell DGV (2015): A network of amygdala connections predict individual differences in trait anxiety. Hum Brain Mapp. 36: 4819–4830. [PubMed: 26769550]
87. He Y, Xu T, Zhang W, Zuo XN (2016): Lifespan anxiety is reflected in human amygdala cortical connectivity. Hum Brain Mapp. 37: 1178–1193. [PubMed: 26859312]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

88. Hadjikhani N, Ward N, Boshyan J, Napadow V, Maeda Y, Truini A, et al. (2013): The missing link: enhanced functional connectivity between amygdala and visceroceptive cortex in migrain. Cephalagia. 33: 1264–1268.

89. Linnman C, Becerra L, Borsook D (2013): Inflaming the Brain: CRPS a model disease to understand Neuroimmune interactions in Chronic Pain. J Neuroimmune Pharmacol. 8: 547–563. [PubMed: 23188523]

90. Graham AM, Pfeifer JH, Fisher PA, Carpenter S, Fair DA (2015): Early life stress is associated with default system integrity and emotionality during infancy. 1–11.

91. Bluhm RL, Williamson PC, Osuch E a, Frewen P a, Stevens TK, Boksman K, et al. (2009): Alterations in default network connectivity in posttraumatic stress disorder related to early-life trauma. J Psychiatry Neurosci. 34: 187–94. [PubMed: 19448848]

92. Lanius RA, Bluhm RL, Coupland NJ, Hegadoren KM, Rowe B, Théberge J, et al. (2010): Default mode network connectivity as a predictor of post-traumatic stress disorder symptom severity in acutely traumatized subjects. Acta Psychiatr Scand. 121: 33–40. [PubMed: 19426163]

93. Zhou Y, Wang Z, Qin L, Wan J, Sun Y, Su S, et al. (2012): Early altered resting-state functional connectivity predicts the severity of post-traumatic stress disorder symptoms in acutely traumatized subjects. PLoS One. 7: e46833. [PubMed: 23056477]

94. Etkin A, Büchel C, Gross JJ (2015): The neural bases of emotion regulation. Nat Rev Neurosci. 16: 693–700. [PubMed: 26481098]

95. Zhang S, Li C-SR (2014): Functional Clustering of the Human Inferior Parietal Lobule by Whole-Brain Connectivity Mapping of Resting-State Functional Magnetic Resonance Imaging Signals. Brain Connect. 4: 140130070445002.

96. Lewis JW, Wightman FL, Brefczynski JA, Phinney RE, Binder JR, DeYoe EA (2004): Human brain regions involved in recognizing environmental sounds. Cereb Cortex. 14: 1008–1021. [PubMed: 15166097]

97. Kheradmand A, Lasker A, Zee DS (2015): Transcranial magnetic stimulation (TMS) of the supramarginal gyrus: A window to perception of upright. Cereb Cortex. 25: 765–771. [PubMed: 24084127]

98. Balestrini S, Francione S, Mai R, Castana L, Casaceli G, Marino D, et al. (2015): Multimodal responses induced by cortical stimulation of the parietal lobe: A stereo-electroencephalography study. Brain. 138: 2596–2607. [PubMed: 26129769]

99. Herringa RJ, Burghy CA, Fox M, Davidson RJ, Essex MJ (2016): Enhanced fronto-subcortical connectivity following childhood adversity as a protective mechanism against internalizing in adolescence. Biol Psychiatry Cogn Neurosci Neuroimaging. 1: S125–S126.

100. Gee DG, Gabard-Durnam LJ, Flannery J, Goff B, Humphreys KL, Telzer EH, et al. (2013): Early developmental emergence of human amygdala – prefrontal connectivity after maternal deprivation. Proc Natl Acad Sci. 110: 15638–15643. [PubMed: 24019460]

101. Mendle J (2014): Why Puberty Matters for Psychopathology. Child Dev Perspect. 8: 218–222.

102. Mendle J, Leve LD, Van Ryzin MJ, Natsuaki MN (2014): Linking Childhood Maltreatment with Girls' Internalizing Symptoms: Early Puberty as a Tipping Point. J Res Adolesence. 24: 689–702.

103. Kim YS, Leventhal BL, Koh Y-J, Fombonne E, Laska E, Lim E-C, et al. (2011): Prevalence of Autism Spectrum Disorders in a Total Population Sample. Am J Psychiatry. 168: 904–912. [PubMed: 21558103]

104. Rice F, Harold GT, Boivin J, Van Den Bree M, Hay D, Thapar A (2010): The links between prenatal stress and offspring development and psychopathology: Disentangling environmental and inherited influences. Psychol Med. 40: 335–342. [PubMed: 19476689]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

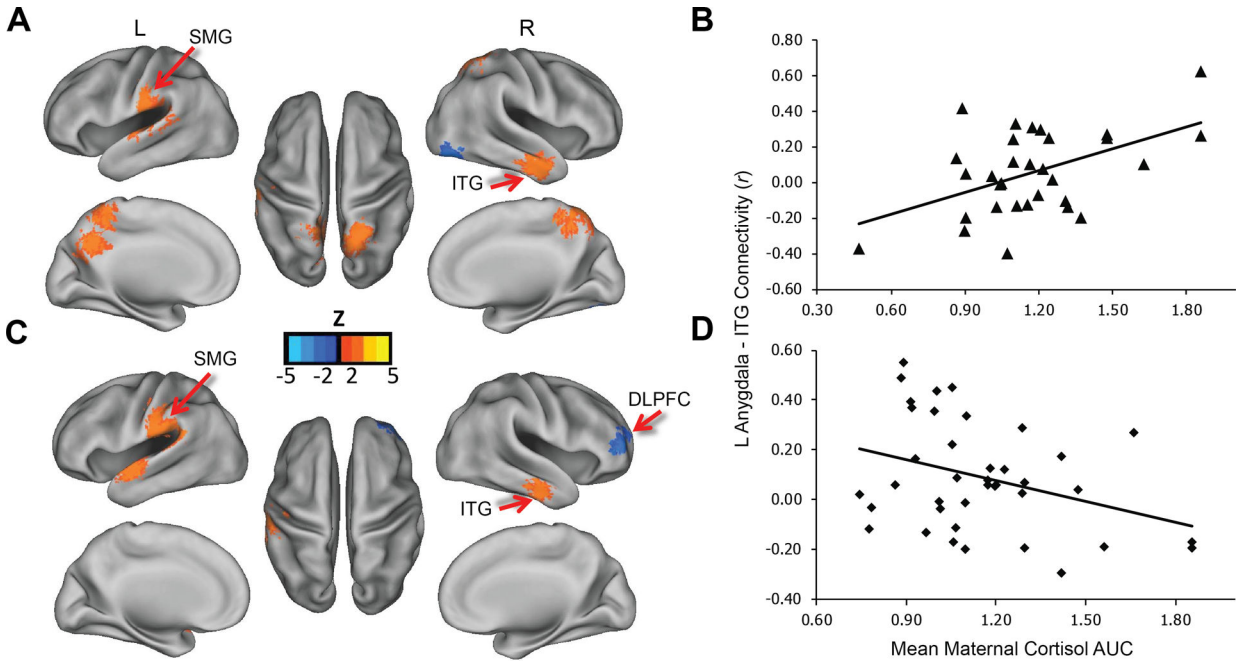

Figure 1.

The interaction between maternal cortisol during pregnancy and infant sex relates to neonatal amygdala connectivity

Note. SMG=supramarginal gyrus; STG=superior temporal gyrus; DLPFC=dorsolateral prefrontal cortex; ITG=inferior temporal gyrus. The association between maternal cortisol during pregnancy and whole brain left (Panel A) and right (Panel C) neonatal amygdala connectivity is moderated by infant sex (N=70). Panels A and C show brain regions for which connectivity of the neonatal amygdala is altered in relation to the interaction between maternal cortisol and infant sex. Scatter plots demonstrate an example of probing the maternal cortisol-infant sex interaction for a specific connection (left amygdala-ITG). For females (Panel B), maternal cortisol evidences a positive association with the strength of this connection (n=31, r= .490, p =.005). For males (Panel D), maternal cortisol evidences a negative association with the strength of this connection (n=39, r= −.342, p =.033). See Table 1 for direction and statistical significance of the bivariate correlations between maternal cortisol and each connection for males and females separately.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

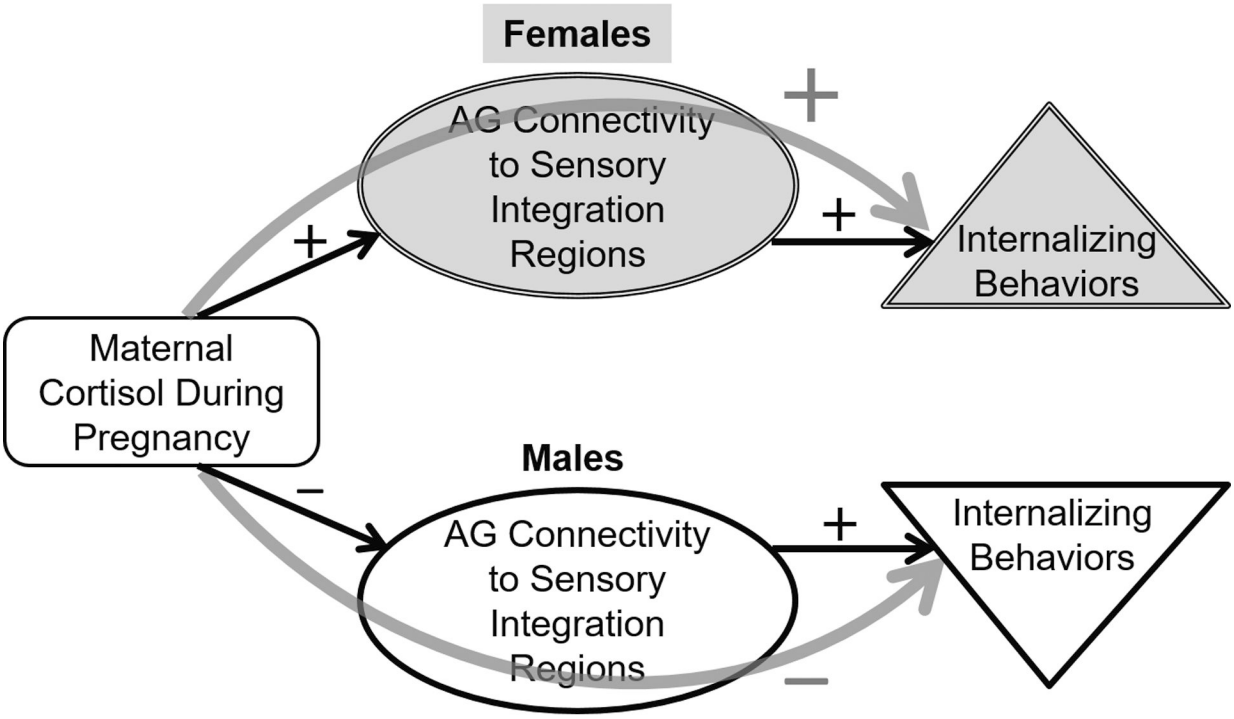

Figure 2.

Conceptual model representing sex-specific associations between maternal cortisol during pregnancy and offspring internalizing behaviors via neonatal amygdala connectivity

Note. The direct effect of maternal cortisol on child internalizing behavior was significant only for females. This model is based on the moderated mediation analysis, which identified significant indirect paths for females and males. Negative or positive sign indicates direction of effect for each association. Grey arrows represent mediation (indirect) effect. For females, higher maternal cortisol concentrations during pregnancy are associated with higher levels of internalizing behaviors at 24-months-of-age via stronger neonatal amygdala-SMG connectivity. For males, higher maternal cortisol during pregnancy is associated with lower levels of internalizing behaviors via weaker neonatal amygdala-SMG connectivity.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1.

Demographics (n = 70)

Characteristic	Value
Maternal Age in First Trimester, Years	28.3 (5.40)
Infant Age, Weeks	
Gestational Age at Birth, Weeks	39.30 (1.39)
Age at MRI Data Collection, Years	3.65 (1.72)
Infant Sex	
Male	62.5
Female	37.5
Race/Ethnicity	
Caucasian non-Hispanic	42.6
African American non-Hispanic	2.13
Asian non-Hispanic	10.6
Multiracial Non-Hispanic	10.6
Caucasian Hispanic	29.8
Asian Hispanic	2.13
Multiracial Hispanic	2.13
Highest Level of Maternal Education	
High School or Test Equivalent	10.4
Vocational School or Some College	50.0
Associate Degree	4.20
Bachelor- or Graduate-Level Degree	35.5
Gross Annual Household Income	
<$15,000	6.38
$15,000–$29,999	19.1
$30,000–$49,999	29.8
$50,000–$100,000	36.2
>$100,000	8.51

Values are mean (SD) or %.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 2.

Mean (SD) of Maternal Cortisol AUC and Gestational Age at Each Collection

	First Trimester	Second Trimester	Third Trimester
Gestational Age, Weeks	12.80 (1.77)	20.50 (1.40)	30.30 (1.26)
Cortisol AUC	2.93 (1.62)	3.25 (1.64)	4.11 (1.68)

Cortisol AUC was log transformed prior to analyses.

AUC, area under the curve.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 3.

The Interaction Between Maternal Cortisol During Pregnancy and Infant Sex Is Prospectively Associated With Neonatal Amygdala Connectivity

Region	Hem	x	y	z	Z	Female Infants	Male Infants
Right Amygdala							
SMG	L	−56	−35	20	2.97	$_{+}c$	$_{-}a$
	L	−51	−25	26	2.88	$_{+}a$	$_{-}c$
STG	L	−48	−7	−7	2.85	$_{+}a$	$_{-}c$
	L	−58	−4	−4	2.65	$_{+}a$	$_{-}c$
ITG	R	52	−17	−24	2.84	$_{+}b$	$_{-}c$
DLPFC	R	38	49	15	−2.67	$_{-}b$	$_{+}b$
	R	31	50	24	−2.50	−	$_{+}c$
Left Amygdala							
ITG	R	52	−7	−23	3.11	$_{+}c$	$_{-}b$
Precuneus/superior parietal	R	16	−54	55	3.05	$_{+}b$	$_{-}b$
SMG	L	−52	−21	23	3.03	$_{+}c$	$_{-}b$
Precuneus	L	−8	−59	32	2.92	$_{+}a$	$_{-}c$
	L	−10	−49	51	2.79	$_{+}c$	$_{-}b$
STG	L	−50	−24	4	2.69	$_{+}b$	$_{-}a$
	L	−47	−14	−1	2.67	$_{+}c$	−
	L	−52	−37	15	2.60	$_{+}b$	$_{-}b$
Fusiform gyrus	R	40	−76	−15	−2.83	$_{-}b$	$_{+}b$

Regions are in descending order based on highest *Z* value. The Pearson correlation between mean maternal gestational cortisol area under the curve and the extracted connection between the amygdala and each identified region was examined separately for female vs. male infants. The direction (positive or negative) and statistical significance of the correlation is indicated for female and male infants, respectively, in the last two columns.

DLPFC, dorsolateral prefrontal cortex; Hem, hemisphere; ITG, inferior temporal gyrus; L, left; R, right; SMG, supramarginal gyrus; STG, superior temporal gyrus.

[a] $p < .10$.

[b] $p < .05$.

[c] $p < .01$.