Neuroendocrine and immune markers of maternal stress during pregnancy and infant cognitive development

Running Title: Antenatal stress and cognitive development

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ABSTRACT

Antenatal exposure to maternal stress is a factor that may impact on offspring cognitive development. While some evidence exists of an association between maternal antenatal depressive or anxiety symptoms and infants' cognitive outcomes, less is known about the role of biological indices of maternal antenatal stress in relation to infant cognitive development. The current study investigated the association between maternal depressive and anxiety symptoms, stress and inflammatory markers during pregnancy and infant's cognitive development in a sample of 104 healthy pregnant women (mean gestational age=34.76; SD=1.12) and their 12-week-old infants (mean postnatal weeks=11.96; SD=1.85). Maternal depressive and anxiety symptoms were evaluated during pregnancy, alongside measurements of serum Interleukin-6 (IL-6), C-Reactive Protein (CRP), salivary cortisol and alpha amylase (sAA) concentrations. Infant cognitive development, maternal caregiving and concurrent anxiety or depressive symptoms were assessed 12 weeks after delivery. Hierarchical linear regressions indicated that higher maternal diurnal cortisol and CRP levels were independently associated with lower infant cognitive development scores, while adjusting for infant gender and gestational age, maternal IQ, caregiving, depressive or anxiety symptoms. Though correlational, findings seem suggestive of a role for variation in maternal biological stress signals during pregnancy in influencing infants' early cognitive development.

KEYWORDS: Pregnancy; Depression; Anxiety; Stress; Cortisol; Inflammation; Cognitive development.

MAIN TEXT

1. Introduction

Healthy cognitive development is an important contributor to an individual's long-term life chances, influencing future academic performance, social competence and mental health (e.g., Peet et al., 2015; Gale, Hatch, Batty, & Deary, 2009). Although cognitive development shows substantial genetic influence (e.g., Kirkpatrick, McGue, Iacono, Miller, & Basu, 2014), there has been a growing appreciation of the role of early environmental exposures in shaping brain development and influencing cognitive outcomes, with increasing attention directed toward prenatal exposures (Grossman et al., 2003). In particular, antenatal exposure to maternal stress is one factor that may influences offspring development across several domains, including the cognitive domain (Van den Bergh et al., 2017). Several community-based studies have investigated the effects of maternal psychological distress experienced during pregnancy, such as depressive or anxiety symptoms, on children's cognitive development (reviewed in Van den Bergh et al., 2017). Antenatal maternal depression has been shown to be associated with poorer infant cognitive development in a number of studies (e.g., Deave, Heron, Evans, & Emond, 2008; Koutra et al., 2013; Evans et al., 2012), although positive associations (Di Pietro, Novak, Costigan, Atella, & Reusing, 2006), as well as null associations (Tse et al., 2010; Nulman et al., 2012; Keim et al., 2011) have also been reported. Likewise, maternal anxiety during pregnancy was associated with lower cognitive scores in offspring in some studies (e.g., Ibanez et al., 2015; Keim et al., 2011; Davis & Sandman, 2010), though not all (Grant, McMahon, Reilly, & Austin, 2010; Plamondon et al., 2015). A recent meta-analysis indicates a very modest negative association ($r=-0.05$), between maternal antenatal stress or anxiety and infant cognitive development, although the effect size varied widely across studies and was stronger for objective indices of maternal stress (e.g., major life events) and for retrospective assessment of maternal antenatal stress (Tarabulsy et al., 2014). Thus, while the association between maternal antenatal anxiety or depression and child cognitive development has been well investigated, the weight of supporting evidence is not yet convincing.

Biological measures of maternal stress might better reflect not only the women's experience of stress during pregnancy but also directly assess the potential biological mechanisms involved that influence fetal development. However, to date, the influence of variation in biological indices of maternal antenatal stress on offspring's cognitive development has received relatively limited investigation.

Cortisol, the end product of the Hypothalamic-Pituitary-Adrenal (HPA) axis, has been the most studied biomarker in relation to the effects of antenatal maternal stress on fetal development. Cortisol plays an essential role in supporting fetal growth and brain development (Lupien, McEwen, Gunnar, & Heim, 2009). However, prenatal exposure to excessive glucocorticoid levels, both synthetic and endogenous, during sensitive developmental periods, can have detrimental effects on fetal brain development, especially in regions rich in cortisol receptors, and can interfere with synaptogenesis and neurotransmitter function (Coe & Lubach, 2005). Fetal cortisol exposure is regulated by the activity of the placental enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which provides partial protection for the fetus from maternal cortisol (Gitau, Cameron, Fisk, & Glover, 1998). It has been hypothesized that stress-linked elevations in maternal cortisol or impairment of the activity of 11β-HSD2, might lead to more cortisol passing through the placenta and directly affecting fetal development (O'Donnell and Meaney, 2017). Few studies have examined the association between endogenous maternal cortisol across gestation and infants' cognitive outcomes and these have yielded mixed findings. Higher maternal cortisol in late pregnancy was associated with lower infant cognitive scores at 3 months (Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2003), 17 months (Bergman, Sarkar, Glover, & O'Connor, 2010) and 7 years (LeWinn et al., 2009), but one study found no association (assessed at 8 months, see Huizink et al., 2003). Davis and Sandman (2010) also reported a negative association between higher maternal cortisol early in gestation and lower mental development at 12 months, but not at 3 and 6 months, and, to complicate the picture further, a positive association was found when maternal cortisol was assessed in late pregnancy. Furthermore, higher maternal cortisol in late pregnancy, but not earlier, predicted greater cortical thickness in frontal regions and better cognitive functioning in the children at 6-9 year of age (Davis, Head, Buss, & Sandman, 2017).

Significant differences in how cortisol is assessed across studies is likely important in explaining these inconsistent findings. Collecting multiple diurnal cortisol measures is generally thought to better reflect maternal stress experience (Harville et al., 2009) and more reliably estimate the total diurnal cortisol output (Adam and Kumari, 2009). However, multiple cortisol measurements have not been employed in many previous studies (exception was one small study (N=30) from Haselbeck et al., 2017).

Very little is known about the influence of alternative biomarkers of maternal antenatal stress on offspring. For example, it has been hypothesized that stress-related activation of the Sympathethic Nervous System (SNS) might affect utero-placental blood flow (Merlot, Couret, & Otten, 2008) and, indirectly, foetal development (Rakers et al., 2017). Salivary alpha amylase (sAA), a non-invasive marker of SNS activity (Nater & Rohleder, 2009), has been found to be associated with infant birth weight (Nazzari et al., 2019; Giesbrecht et al., 2015), which in turn, is a well-established predictor of later cognitive abilities (e.g. Shenkin, Starr, & Deary, 2004). However, the association between maternal antenatal sAA levels and infant cognitive development has not yet been investigated.

Likewise, it is now well-established that stress is associated with changes in immune system functioning, including increased levels of inflammatory markers such as Interleukine-6 (IL-6) and C-reactive protein (CRP), both in non-pregnant (e.g., Segerstrom & Miller, 2004) and pregnant (e.g. Christian et al., 2012) samples. Furthermore, maternal antenatal inflammation is increasingly recognized as a factor that may influence offspring development (Gumusoglu & Stevens, 2019). Neverthless, research in humans is at an early stage (Nazzari & Frigerio, 2019). Elevated maternal IL-6 or CRP levels during pregnancy have been linked with adverse pregnancy and birth outcomes (Christian, 2012) and with new-born functional and structural brain alterations (e.g. ,Graham et al., 2018; Rudolph et al., 2018). Only two studies have examined the role of maternal antenatal inflammation on infant cognitive development. Rasmussen and colleagues (2019) found that higher maternal IL-6 levels were associated with poorer cognitive development at 12-months, whereas Spann and colleagues (2018) reported a positive association between

maternal IL-6 and CRP levels in late pregnancy and 14-month-olds' cognitive development in a small sample of 36 adolescent mothers.

1.1 Current study

There is a growing recognition for a role of maternal antenatal stress signals in influencing fetal development and for possible long-lasting effects of such exposures on cognitive outcomes. However, existing studies have been characterized by a number of limitations. First, few prospective studies have combined multiple psychological and biological stress measures and most studies have focused on cortisol as a biomarker of maternal stress experience, while alternative biomarkers of stress or inflammation have been largely neglected. We therefore have only a limited picture of the spectrum of biological processes that may be involved in antenatal stress effects on cognitive development, or the relative independence or degree of overlap between different biological indicators and their association with cognitive outcomes. Secondly, almost all existing studies rely on single maternal cortisol sampling which does not allow for a reliable estimate of overall diurnal cortisol production. Third, available results are often beset by inconsistent inclusion of key confounders in the analyses. Specifically, because cognitive abilities are substantially heritable (e.g., Kirkpatrick et al., 2014) and may also correlate with social risk factors like low parental education, it is important to control for the role of maternal IQ, something that is rarely done in the field. Likewise, despite evidence indicating an important role for maternal early caregiving on infants' cognitive development (e.g., Mills-Koonce et al., 2015), existing studies rarely account for the quality of early post-natal mother-infant relationship, thus limiting the inferences that can be made about programming effects of the prenatal environment.

The current study contributes to the literature by investigating the influence of variation in maternal self-reported depressive and anxiety symptoms, stress hormones and inflammatory markers in late pregnancy on infant cognitive outcomes, controlling for maternal IQ, postnatal symptomatology and caregiving. We hypothesized that infants born to mothers with higher depressive or anxiety symptoms during pregnancy would show poorer cognitive performance than infants of mothers with lower symptoms. Furthermore, we predicted that higher maternal antenatal

diurnal cortisol would be related to lower cognitive scores. We made no a priori predictions for an association between maternal sAA levels or inflammatory markers and infants' outcomes due to limited available literature.

2 Material and methods

2.1 Participants and procedure

One hundred and ten women were recruited between 30th and 33rd weeks of gestation as part of the Effects of Depression on Infants (EDI) Study and followed longitudinally until 12 weeks post-delivery. Prenatal inclusion criteria were: aged 18-45 years, normotensive, with singleton uncomplicated pregnancy, non-smoker, not affected by any disease or taking any chronic medications, and with no current substance/alcohol abuse problems or psychiatric disorders (except for depression and anxiety) as ascertained through the *Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID-I;* First, Spitzer, Gibbon, & Williams, 2002). Additionally, only healthy full term (or late preterm >35 weeks) infants were included in the current study. Six women were excluded because of: intrauterine death (N=1), newborn health problems (N=2) and missing infant developmental data (N=3), thus the final sample consisted of 104 mother-infant dyads. Women were mostly Italian (97.2%), middle-upper class (87.5%) and primiparous (91.3%). Infants (51% males) were mostly delivered vaginally (81.7%) at 39.44 mean gestational age (SD=1.25). Women who were excluded from the postnatal assessment did not differ from participants on any demographic variables, depression or anxiety scores. Around 17% of women scored equal or above the cut-off of 10 at the Edinburgh Postnatal Depression Scale (EPDS, Benvenuti et al., 1999) and 24% of women scored equal or above the cut–off of 40 at the state anxiety subscale of the State-Trait Anxiety Inventory (STAI-S, Spielberger et al., 1970). Furthermore, 4.8% of women had a major depressive episode and 2,9% had an anxiety disorder during pregnancy as ascertained through the SCID-I.

Maternal depressive and anxiety symptoms were evaluated between 34-36 gestational weeks (mean gestational age=34.76; SD=1.12), jointly with maternal cognitive function and

biomarkers levels. Infant cognitive development, maternal caregiving and concurrent anxiety or depressive symptoms were assessed 12 weeks after delivery (mean postnatal weeks=11.96; SD=1.85).

The Ethics Committee of Scientific Institute Eugenio Medea and of University College London approved the study protocol and all mothers signed an informed written consent to participate.

2.2 Maternal assessment

Psychological distress. Depressive symptoms were evaluated through the Italian version (Benvenuti et al., 1999) of the 10-item Edinburgh Postnatal Depression Scale (EPDS; Cox, Holden, & Sagovsky, 1987), the most widely used self-report questionnaire to assess perinatal depressive symptoms on a 4-point Likert scale. Anxiety symptoms were evaluated on a 4-point Likert scale through the Italian version (Pedrabissi & Santinello, 1989) of the 20-item state anxiety subscale of the State-Trait Anxiety Inventory (STAI) (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1970).

Cognitive function was evaluated through the Raven's Standard Progressive Matrices (RSPM, Raven & Court, 1998), a widely employed test that assesses non-verbal general cognitive ability through 60 multiple choice items. Test time was limited to 30 minutes.

Stress and inflammatory biomarkers. All women provided six saliva samples on two consecutive days upon awakening, 30 minutes post-waking and before going to bed. Samples were assayed for cortisol in duplicate using a competitive high sensitivity enzyme immunoassay kit (Expanded Range High Sensitivity Cortisol EIA Kit, Salimetrics) and for sAA using a kinetic enzyme assay kit (Salimetrics α-Amylase Kinetic Enzyme Assay Kit). One 30-min post-waking sample collected more than one hour from awakening was excluded from analyses (e.g., O'Connor et al. 2014). Daily average cortisol and sAA were calculated as the area under the curve (AUCg) using the trapezoid method with respect to the ground (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003) for each day separately and the mean of the two days was used as the two values were highly correlated ($r=0.58$, $p<.001$, for cortisol, $r=0.74$, $p<.001$ for sAA). Antecubital venous blood samples were collected in the morning. Serum CRP and IL-6 concentrations were

assayed in duplicate by using Quantikine High Sensitivity ELISA kits (R&D Systems Europe, LTD). Ninety-one women out of 104 (87.5%) agreed to blood sampling during pregnancy. For further details see Nazzari et al., 2019.

Caregiving behaviors were evaluated using the Emotional Availability (EA) Scales, Infancy/Early Childhood Version (4th edition, Biringen, 2008) during a 15 minute-videotaped free-play session. The EA scales comprise four maternal parenting dimensions (Sensitivity, Structuring, Non-intrusiveness and Non-hostility) rated on a 7-point scale. As the four maternal EA scales were moderately inter-correlated (rs=0.50–0.73), they were standardized and summed to create an overall index of maternal EA (Cronbach's α=.85) as previously reported (Taylor-Colls & Fearon, 2015). Two trained clinical psychologists independently coded the videotaped interactions after the end of data collection. Both raters were blind to prenatal and postnatal data. A subsample of 20 (19.5%) randomly chosen dyads were coded by both raters and showed high inter-rater consistency with intra-class correlation (ICC) coefficients ranging from .75 to .91 and a mean ICC of .84 ($p<.001$).

2.3 Infant assessment

Cognitive development was assessed through the Bayley Scales of Infant and Toddler Development – Third Edition (Bayley III, Bayley 2006), a well-standardized and widely employed developmental assessment, by a trained clinical psychologist blind to prenatal data. The Cognitive Scale measures sensorimotor integration, attention, habituation and memory, as well as interest in and understanding of the environment. Age-standardized composite scores were calculated by using test norms (mean=100; SD=15).

2.4 Statistical analyses

Variables were first examined for outliers and skewness. The distributions of the biological markers were positively skewed even after removing samples greater than 3 SD from the mean (n=7 samples for cortisol, n=4 for sAA, n=3 for IL-6), thus measures were natural log transformed prior to analysis to approximate normal distributions. Potential confounders of the relationship

between maternal prenatal variables and infants' outcomes were examined through preliminary Pearson correlations and univariate analysis of variance (ANOVA). Separate hierarchical regression analyses were performed to evaluate the effects of maternal variables on infant's cognitive scores. Covariates were entered in the first step. Maternal depression/anxiety scores were entered in a second step to assess the independent effect of prenatal maternal self-reported symptoms on infant outcomes, while biological markers concentrations were included in a third step to evaluate the unique contribution of maternal stress biomarkers to infant outcomes. Non-linearity of the observed associations was examined by the inclusion of a quadratic term within the regression models. As maternal depression and anxiety were highly correlated both prenatally ($r=0.66$, $p<.001$) and postnatally ($r=.58$, $p<.001$), they were entered separately in subsequent analyses in order to avoid multicollinearity. Analyses including maternal anxiety rather than depression yielded comparable results and are reported in Supplementary Table 1. Lastly, we repeated analyses after excluding women with CRP levels above 10mg/L (N=5), since such levels could be due to subclinical acute infections and not reflect chronic inflammation (e.g. Wu et al., 2002; Belo et al., 2005).

The final sample size was 104 for analyses on the association between maternal stress markers (i.e. cortisol and sAA) and infant outcomes and 91 for analyses on the association between maternal inflammatory markers (i.e. IL-6 and CRP) and infant outcomes. Statistical analyses were performed using SPSS 24.

3 Results

3.1 Descriptive analyses and confounders

Descriptive statistics for all study variables are presented in Table 1 using raw values. As displayed in Table 2, preliminary unadjusted bivariate correlations among study variables showed weak associations between maternal depressive or anxiety symptoms and biomarkers levels (r's range= -.14-.19). In contrast, a modest negative association was found between maternal antenatal cortisol and infants' cognitive scores (r=-.26).

Variables examined as potential confounders included sociodemographic factors (i.e., maternal age, education, SES, parity, infants' age), health-related factors (i.e., mode of delivery, length of labor, gestational age, birth weight, head circumference, actual weight, postnatal smoke exposure, breastfeeding versus formula-feeding), maternal EA and IQ. Gestational age at birth, maternal EA and IQ were modestly associated with infants' cognitive performances (r's range=.21-.24), thus they were retained as covariate in subsequent models. As sex-differences in the association between prenatal maternal stress and infants' cognitive outcomes have been reported (e.g., Loomans et al., 2012), all subsequent analyses controlled for infant gender. Furthermore, as an association between maternal postnatal depression and infant cognitive outcomes has been reported (Liu et al., 2017), maternal concurrent symptoms were included as confounders in all regression models. Models were performed both with and without the inclusion of postnatal symptoms. Adjusting for maternal depressive or anxiety symptoms at 3 months did not affect the statistical significance and direction of the association between prenatal maternal variables and infant outcomes in any of the analyses presented below.

3.2 Prenatal maternal influences and infant's cognitive development

As shown in Table 3, hierarchical regression models revealed significant associations between higher maternal antenatal diurnal cortisol levels and lower cognitive scores at 3-months (β=-.37, t= -3.87, p<.001), while adjusting for infant gender, gestational age, maternal IQ, EA, pre- and postnatal depression. Similarly, maternal antenatal CRP levels were significantly negatively associated with infant cognitive development (β=-.22, t= -2.05, p=.04). In contrast, no effect of maternal antenatal depression on infant cognitive scores was found.

Supplementary analyses indicated that the direction and statistical significance of the association between maternal cortisol and CRP levels during pregnancy and infant cognitive development remained substantially unchanged once all the biomarkers were included in a final regression model (respectively, β=-.32, t= -2.96, p=.004; β=-.23, t= -2.14, p=.04), thus suggesting that both biomarkers contribute significantly and independently to infant cognitive scores. Furthermore, in order to examine the existence of non-linear associations between maternal

biomarkers and infant cognitive outcomes, we repeated all the analyses including a quadratic term for maternal cortisol and CRP. When added to the models, centered second order polynomials of cortisol or CRP were not significantly associated with infant cognitive scores (respectively, β= -.004, t= -0.04, p=.97; β=.11, t=0.98, p=.33), thus indicating that the observed association between maternal biomarkers and infant cognitive outcomes was linear. Lastly, the main effect of maternal antenatal CRP levels on infant cognitive scores (β= -.24, t= -2.26, p=.027) remained significant when women with possible acute infections (CRP > 10.0 mg/L) were excluded.

4. Discussion

The current study sought to extend existing literature on the programming role of maternal antenatal stress in infant cognitive development by combining multiple psychological and biological stress markers and controlling for key confounders of the association. Normative variations in maternal diurnal cortisol and inflammation during pregnancy were independently and linearly associated with individual differences in 3-month-olds' cognitive development. Importantly, the observed associations were found when controlling for maternal IQ, caregiving, infant gender and gestational age and were independent of antenatal and postnatal anxiety and depression. In contrast, maternal depressive or anxiety symptoms were not significantly associated with infants' cognitive scores.

In line with our predictions, we observed lower cognitive scores in infants whose mothers had higher levels of antenatal diurnal cortisol. Using multiple diurnal sampling, the present results support and extend the limited available literature linking higher levels of maternal endogenous cortisol in late pregnancy with poorer infant cognitive development (Huiznik et al., 2003; Bergman et al., 2010; LeWinn et al., 2009). The pathways underlying the observed association are still unclear. Though essentially correlational in nature, findings are consistent with animal evidence of neuro-motor and learning deficits in prenatally stressed offspring (Owen, Andrews, & Matthews, 2005) and with studies linking prenatal glucocorticoid administration to reduced cognitive abilities and cortical thinning (Davis et al., 2013), and might suggest a role of maternal antenatal cortisol in influencing fetal brain development. As the expression of the 11β-HSD2 enzyme is reduced in the

last stages of gestation (Murphy, Smith, Giles, & Clifton, 2006), even mild elevations in maternal cortisol in late pregnancy might affect fetal brain development, particularly in regions sensitive to excessive levels of glucocorticoids such as the hippocampus (Noorlander et al., 2006), potentially leading to cognitive and behavioral alterations. Furthermore, maternal cortisol might influence fetal brain development indirectly by affecting placental blood vessel tone and leading to reduced utero-placental blood flow. However, the observed association could also be explained by shared predictors of prenatal maternal cortisol and infant development, such as maternal diet or genetic factors, which were not measured in the current study.

We also obtained evidence of an association between higher maternal CRP levels in late pregnancy and poorer infant cognitive development. Accumulating evidence from animal models suggests that maternal antenatal inflammation can influence the earliest stages of brain development and be responsible of altered neurodevelopmental outcomes in the offspring (reviewed in Gumusoglu & Stevens, 2019). More specifically, preclinical studies of rodents and non-human primates have shown that activating the maternal immune system during pregnancy can affect brain myelination (Cai, Pan, Pang, Evans, & Rhodes, 2000), alter a widespread set of brain regions, such as the hippocampus, prefrontal cortex, mid-temporal and parietal lobe (e.g., Patterson, 2002; Golan et al., 2005; Bland et al., 2010; Short et al., 2010) and lead to behavioral disturbances, including learning, attention and memory deficits (e.g., Bilbo et al., 2008; Patterson, 2009). Our findings of an association between higher third trimester maternal inflammation and poorer infant cognitive outcomes are in line with this body of evidence. However, it is worthwhile noting that while preclinical studies traditionally defined maternal immune activation as measured levels of inflammatory markers exceeding normal range, the association reported here remained strong and significant once women with CRP levels above 10 mg/L, possibly indicating acute infections (e.g. Wu et al., 2002), were excluded, thus suggesting that even modest and subthreshold variations in maternal inflammation might be associated with individual variation in early post-natal cognitive function in humans.

The current results converge with initial evidence linking maternal antenatal inflammation with poorer neurobehavioral function at 6 days of age (Osborne et al., 2018) and poorer cognitive

development at 12 months (Rasmussen et al., 2019). Interestingly, structural and functional brain alterations (Graham et al., 2018; Rasmussen et al., 2019) and smaller head circumference at birth (Nazzari et al., 2019) have been found in newborns prenatally exposed to higher maternal IL-6 levels, thus possibly suggesting that naturally occurring variations in antenatal levels of maternal inflammation could influence fetal brain development, although the mechanisms underlying this effect are still unknown. It is noteworthy that, despite the fact that both CRP and IL-6 are biomarkers of chronic or systemic low-grade inflammation (Rohleder, Aringer, & Boentert, 2012) and were correlated in the current sample, maternal antenatal IL-6 concentrations were not associated with 3-month-olds' cognitive outcomes. These differences might be related to methodological issues, such as differential sensitivity of CRP and IL-6 to processing and storage conditions (Skogstrand et al., 2008). However, they might also be suggestive of distinct inflammatory pathways involved in fetal programming that deserve further investigation. For example, heightened maternal CRP levels could interfere with fetal growth and brain development by contributing to vascular and placental dysfunction (Ernst et al., 2011) or by directly altering offspring's synaptic connectivity (Canetta et al., 2014). Secondly, higher CRP levels may indicate poor maternal health (Wium-Andersen & Nielsen, 2013), which can itself influence offspring brain development (Chittleborough, Lawlor, & Lynch, 2012) and, therefore, could potentially account for the current findings. Third, animal studies indicate that epigenetic mechanisms could mediate the impact of prenatal maternal inflammation on fetal development (Kundakovic & Jaric, 2017).

Current results based on a screened, physically healthy population of pregnant mothers suggested that the association between maternal antenatal cortisol or CRP and infant cognitive developmental outcomes behaved in a linear fashion, in line with previous studies showing a dose-response relationship across the range between prenatal stress and child outcomes (e.g., MacKinnon, Kingsbury, Mahedy, Evans & Colman, 2018; Kingston et al., 2018; O'Connor, Heron, Golding, Beveridge, & Glover, 2002). Furthermore, it is worth mentioning that the effects we reported were independent of a range of maternal pre- and postnatal factors (i.e. concurrent depressive or anxiety symptoms, quality of caregiving, IQ), and were not fully explained by infant factors (i.e. gender or gestational age). These results strengthen the hypothesis that prenatal

maternal stress biology can independently predict individual differences in early infant cognitive development. Future studies should investigate the extent to which the observed associations are independent of genetic influences, which are likely to confound the associations among prenatal factors and child outcomes, by adopting genetically informed designs (Rice, Langley, Woodford, Smith, & Thapar, 2018).

Some non-significant findings are noteworthy. In contrast to our initial hypotheses, maternal prenatal depressive or anxiety symptoms were not significantly associated with 3-month-olds' cognitive development. It is possible that the relatively mild levels of maternal depression or anxiety symptoms in the current low-risk sample could have limited our ability to detect a significant association. However, it is worth mentioning that null findings have also been reported in clinical (Osborne et al., 2018; O'Leary et al., 2019) and high-risk samples (Bandoli et al., 2016). Additionally, we cannot rule out that the exclusion of pregnant women with chronic medical problems or obstetric complications, resulting in a highly selected sample, might have biased the observed associations. Alternatively, the effects of maternal prenatal depression or anxiety on cognitive outcomes might emerge later in development (e.g., Koutra et al., 2013; Lin et al., 2017; Evans et al., 2012). Interestingly, in line with several studies (e.g., Davis & Sandman, 2010; Voegtline et al., 2013), self-reported anxiety or depressive symptoms were also unrelated to biomarkers levels. This underscores the importance of combining subjective and objective measures of maternal antenatal stress in order to capture the maternal experience (O' Donnell & Meaney, 2017).

We also found no associations between maternal antenatal sAA levels and infant cognitive outcomes. Evidence for a role of maternal antenatal SNS in fetal programming is scarce and, to our knowledge, no published data exists on the link between prenatal maternal sAA levels and infant cognitive measures. Further replication of this finding in different cohorts is needed before strong conclusions can be drawn about the association between sAA and infant cognitive outcomes.

4.1 Limitations

The interpretation of our findings should be made with due caution as a result of several limitations. First, the results were based on a relatively small middle-high SES community sample of healthy women and infants, thus limiting generalizability to different and high-risk populations. Secondly, our investigation was limited to one time point in late pregnancy, so the observed associations might not extend to other gestational periods. Third, maternal salivary samples were collected at home and compliance with the protocol was not objectively measured. Fourth, although findings of a prospective association between maternal antenatal factors and offspring outcome were in the expected direction and it is tempting to interpret them as suggestive of causative biological pathways, they are correlational analyses and no causal conclusions should be drawn.

4.2 Conclusion

The current findings support the view that variations in maternal biological stress signals during pregnancy could influence infants' early cognitive development and encourage further investigation into inflammatory mechanisms that have been largely neglected until recently. Early performance on the Bayley cognitive scale have been associated with IQ in later childhood (e.g., Bode, D'Eugenio, Mettelman, & Gross, 2014) which, in turn, has been related to later academic performance and the occurrence of behavioral problems (Boutwell, Helton, Vaughn, & Kavish, 2017). Thus, the current results might indicate one possible pathway through which prenatal stress exposure could impact long-term development, and hence deserve further investigation.

REFERENCES

Adam, E. K., & Kumari, M. (2009). Assessing salivary cortisol in large-scale, epidemiological research. *Psychoneuroendocrinology*, *34*(10), 1423-1436. https://doi:10.1016/j.psyneuen.2009.06.011

Bandoli, G., Coles, C. D., Kable, J. A., Wertelecki, W., Granovska, I. V., Pashtepa, A. O., ... & CIFASD. (2016). Assessing the independent and joint effects of unmedicated prenatal depressive symptoms and alcohol consumption in pregnancy and infant neurodevelopmental outcomes. *Alcoholism: Clinical and Experimental Research*, *40*(6), 1304-1311. https://doi: 10.1111/acer.13081.

Bayley, N. (2006). Bayley scales of infants and toddler development (3rd ed.). San Antonio, TX US: Harcourt Assessment Inc.

Belo, L., Santos-Silva, A., Rocha, S., Caslake, M., Cooney, J., Pereira-Leite, L., ... & Rebelo, I. (2005). Fluctuations in C-reactive protein concentration and neutrophil activation during normal human pregnancy. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *123*(1), 46-51.

Benvenuti, P., Ferrara, M., Niccolai, C., Valoriani, V., & Cox, J. L. (1999). The Edinburgh Postnatal Depression Scale: validation for an Italian sample*. Journal of Affective Disorders*, 53(2), 137–141.

Bergman, K., Sarkar, P., Glover, V., & O'Connor, T. G. (2010). Maternal Prenatal Cortisol and Infant Cognitive Development: Moderation by Infant-Mother Attachment. *Biol Psychiatry*, 67(11), 1026–1032. https://doi.org/10.1016/j.biopsych.2010.01.002.I

Bilbo, S.D., Yirmiya, R., Amat, J., Paul, E.D., Watkins, L.R. & Maier, S.F. (2008). Bacterial infection early in life protects against stressor-induced depressive-like symptoms in adult rats. Psychoneuroendocrinology, 33, 261–269. https://doi:10.1016/j.psyneuen.2007.11.008.

Biringen, Z. (2008). Emotional Availability Scales, 4th Edition. Boulder: Colorado State University.

Bland, S.T., Beckley, J.T., Young, S., Tsang, V., Watkins, L.R., Maier, S.F. & Bilbo, S.D. (2010) Enduring consequences of early-life infection on glial and neural cell genesis within cognitive regions of the brain. *Brain Behav Immun* 24, 329–338. https://doi:10.1016/j.bbi.2009.09.012.

Bode, M. M., D'Eugenio, D. B., Mettelman, B. B., & Gross, S. J. (2014). Predictive validity of the Bayley, at 2 years for intelligence quotient at 4 years in preterm infants. *Journal of Developmental & Behavioral Pediatrics*, *35*(9), 570-575. https:// doi: 10.1097/DBP.0000000000000110

Boutwell, B. B., Helton, J., Vaughn, M., & Kavish, N. (2017). The Association of Externalizing and Internalizing Problems with Indicators of Intelligence in a Sample of At-Risk Children. bioRxiv. Retrieved from http://biorxiv.org/content/early/2017/10/28/210500.abstract

Cai, Z., Pan, Z.-L., Pang, Y. I., Evans, O. B., & Rhodes, P. G. (2000). Cytokine induction in fetal rat brains and brain injury in neonatal rats after maternal lipopolysaccharide administration. *Pediatric Research*, 47(1), 64.

Canetta, S., Ph, D., Sourander, A., Surcel, H., Ph, D., Lic, P., … Brown, A. S. (2014). Elevated Maternal C-Reactive Protein is Associated with Increased Risk of Schizophrenia in a National Birth Cohort Sarah. *Am J Psychiatry*, 171(9), 960–968. https://doi.org/10.1176/appi.ajp.2014.13121579.

Chittleborough, C. R., Lawlor, D. A., & Lynch, J. W. (2012). Prenatal prediction of poor maternal and offspring outcomes: implications for selection into intensive parent support programs. *Maternal and Child Health Journal*, 16(4), 909–920. https://doi: 10.1007/s10995-011-0818-5.

Christian, L. M. (2012). Psychoneuroimmunology in pregnancy: immune pathways linking stress with maternal health, adverse birth outcomes, and fetal development. *Neuroscience & Biobehavioral Reviews*, *36*(1), 350-361. https://doi: 10.1016/j.neubiorev.2011.07.005.

Coe, C. L., & Lubach, G. R. (2005). Prenatal origins of individual variation in behavior and immunity. *Neuroscience and Biobehavioral Reviews*, 29 (1 SPEC. ISS.), 39–49. https://doi.org/10.1016/j.neubiorev.2004.11.003

Cox, J. L., Holden, J. M., & Sagovsky, R. (1987). Detection of postnatal depression . Development of the 10-item Edinburgh Postnatal Depression Scale Detection of Postnatal Depression Development of the 10-item Edinburgh Postnatal Depression Scale. *Br J Psychiatry*, 150, 782–786. https://doi.org/10.1192/bjp.150.6.782

Davis, E. P., Head, K., Buss, C., & Sandman, C. A. (2017). Prenatal maternal cortisol concentrations predict neurodevelopment in middle childhood. *Psychoneuroendocrinology,* 75, 56–63. https://doi.org/10.1016/j.psyneuen.2016.10.005

Davis, E. P., & Sandman, C. A. (2010). The timing of prenatal exposure to maternal cortisol and psychosocial stress is associated with human infant cognitive development. *Neuroscience and Biobehavioral Reviews*, 29(1), 3–14. https://doi.org/10.1111/j.1467-8624.2009.01385.x.The

Davis, E. P., Sandman, C. A., Buss, C., Wing, D. A., & Head, K. (2013). Fetal glucocorticoid exposure is associated with preadolescent brain development. *Biological Psychiatry*, 74(9), 647–655. https://doi: 10.1016/j.biopsych.2013.03.009

Deave, T., Heron, J., Evans, J., & Emond, A. (2008). The impact of maternal depression in pregnancy on early child development. *BJOG: An International Journal of Obstetrics & Gynaecology*, *115*(8), 1043-1051. https://doi: 10.1111/j.1471-0528.2008.01752.x.

DiPietro, J. A., Novak, M. F., Costigan, K. A., Atella, L. D., & Reusing, S. P. (2006). Maternal psychological distress during pregnancy in relation to child development at age two. *Child development*, *77*(3), 573-587.

Ernst, G. D. S., de Jonge, L. L., Hofman, A., Lindemans, J., Russcher, H., Steegers, E. A. P., & Jaddoe, V. W. V. (2011). C-reactive protein levels in early pregnancy, fetal growth patterns, and the risk for neonatal complications: the Generation R Study. *American Journal of Obstetrics and Gynecology*, 205(2), 132.e1-12. https://doi: 10.1016/j.ajog.2011.03.049

Evans, J., Melotti, R., Heron, J., Ramchandani, P., Wiles, N., Murray, L., & Stein, A. (2012). The timing of maternal depressive symptoms and child cognitive development: A longitudinal study. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, 53(6), 632–640. https://doi.org/10.1111/j.1469-7610.2011.02513.x

First, M., Spitzer, R., Gibbon, M., & Williams, J. (2002). Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition (SCID-I/P). New York: Biometrics Research.

Gale, C. R., Hatch, S. L., Batty, G. D., & Deary, I. J. (2009). Intelligence in childhood and risk of psychological distress in adulthood: the 1958 National Child Development Survey and the 1970 British Cohort Study. *Intelligence*, *37*(6), 592-599. https://doi.org/10.1016/j.intell.2008.09.002

Giesbrecht, G. F., Campbell, T., Letourneau, N., Kaplan, B. J., Field, C. J., Dewey, D., … Singhal, N. (2015). Sexually dimorphic adaptations in basal maternal stress physiology during pregnancy and implications for fetal development. *Psychoneuroendocrinology,* 56, 168–178. https://doi.org/10.1016/j.psyneuen.2015.03.013

Gitau, R., Cameron, A., Fisk, N. M., & Glover, V. (1998). Fetal exposure to maternal cortisol. *The Lancet*, *352*(9129), 707-708.

Golan, H. M., Lev, V., Hallak, M., Sorokin, Y., & Huleihel, M. (2005). Specific neurodevelopmental damage in mice offspring following maternal inflammation during pregnancy. *Neuropharmacology*, 48(6), 903–917. https://doi.org/10.1016/j.neuropharm.2004.12.023

Graham, A. M., Rasmussen, J. M., Rudolph, M. D., Heim, C. M., Gilmore, J. H., Styner, M., ... & Buss, C. (2018). Maternal systemic interleukin-6 during pregnancy is associated with newborn amygdala phenotypes and subsequent behavior at 2 years of age. *Biological Psychiatry*, *83*(2), 109-119. https://doi: 10.1016/j.biopsych.2017.05.027

Grossman, A. W., Churchill, J. D., Mckinney, B. C., Kodish, I. M., Otte, S. L., & Greenough, W. T. (2003). Experience effects on brain development: possible contributions to psychopathology. *Journal of Child Psychology and Psychiatry*, 1(44), 33–63.

Grant, K. A., McMahon, C., Reilly, N., & Austin, M. P. (2010). Maternal sensitivity moderates the impact of prenatal anxiety disorder on infant mental development. *Early Human Development*, *86*(9), 551-556. https://doi: 10.1016/j.earlhumdev.2010.07.004.

Gumusoglu, S. B., & Stevens, H. E. (2019). Maternal inflammation and neurodevelopmental programming: a review of preclinical outcomes and implications for translational psychiatry. *Biological psychiatry*, *85*(2), 107-121.

Harville, E. W., Savitz, D. A., Dole, N., Herring, A. H., & Thorp, J. M. (2009). Stress questionnaires and stress biomarkers during pregnancy. *Journal of Women's Health*, *18*(9), 1425-1433.

Haselbeck, C., Niederberger, U., Kulle, A., Wache, K., Brauner, E., Gutermuth, M., ... & Siniatchkin, M. (2017). Prenatal maternal distress seems to be associated with the infant's temperament and motor development: an explorative study. *Journal of Neural Transmission*, *124*(7), 881-890. https:// doi: 10.1007/s00702-017-1712-0

Huizink, A. C., Robles de Medina, P. G., Mulder, E. J., Visser, G. H., & Buitelaar, J. K. (2003). Stress during pregnancy is associated with developmental outcome in infancy. *Journal of Child Psychology and Psychiatry*, *44*(6), 810-818.

Keim, S. A., Daniels, J. L., Dole, N., Herring, A. H., Siega-Riz, A. M., & Scheidt, P. C. (2011). A prospective study of maternal anxiety, perceived stress, and depressive symptoms in relation

to infant cognitive development. *Early human development*, *87*(5), 373-380. https://doi: 10.1016/j.earlhumdev.2011.02.004.

Kingston, D., Kehler, H., Austin, M. P., Mughal, M. K., Wajid, A., Vermeyden, L., ... & Giallo, R. (2018). Trajectories of maternal depressive symptoms during pregnancy and the first 12 months postpartum and child externalizing and internalizing behavior at three years. *PloS one*, *13*(4), e0195365. https://doi.org/10.1371/journal.pone.0195365

Kirkpatrick, R. M., McGue, M., Iacono, W. G., Miller, M. B., & Basu, S. (2014). Results of a "GWAS Plus:" General cognitive ability is substantially heritable and massively polygenic. *PloS One,* 9(11), e112390. https://doi: 10.1371/journal.pone.0112390.

Koutra, K., Chatzi, L., Bagkeris, M., Vassilaki, M., Bitsios, P., & Kogevinas, M. (2013). Antenatal and postnatal maternal mental health as determinants of infant neurodevelopment at 18 months of age in a mother-child cohort (Rhea Study) in Crete, Greece. *Social Psychiatry and Psychiatric Epidemiology,* 48(8), 1335–1345. https://doi.org/10.1007/s00127-012-0636-0

Kundakovic, M., & Jaric, I. (2017). The epigenetic link between prenatal adverse environments and neurodevelopmental disorders. *Genes,* 8(3). https://doi.org/10.3390/genes8030104

Laucht, M., Esser, G., & Schmidt, M. H. (1997). Developmental outcome of infants born with biological and psychosocial risks. *Journal of Child Psychology and Psychiatry*, 38(7), 843–853.

Lewinn, K. Z., Stroud, L. R., Molnar, B. E., Ware, J. H., Koenen, K. C., & Buka, S. L. (2009). Elevated maternal cortisol levels during pregnancy are associated with reduced childhood IQ. *International Journal of Epidemiology,* 38(6), 1700–1710. https://doi.org/10.1093/ije/dyp200

Lin, Y., Xu, J., Huang, J., Jia, Y., Zhang, J., Yan, C., & Zhang, J. (2017). Effects of prenatal and postnatal maternal emotional stress on toddlers' cognitive and temperamental development. *Journal of Affective Disorders*, 207, 9–17. https://doi.org/10.1016/j.jad.2016.09.010

Liu, Y., Kaaya, S., Chai, J., McCoy, D. C., Surkan, P. J., Black, M. M., ... & Smith-Fawzi, M. C. (2017). Maternal depressive symptoms and early childhood cognitive development: a meta-analysis. *Psychological medicine*, *47*(4), 680-689. https://doi: 10.1017/S003329171600283X.

Loomans, E. M., van der Stelt, O., van Eijsden, M., Gemke, R. J. B. J., Vrijkotte, T. G. M., & Van den Bergh, B. R. H. (2012). High levels of antenatal maternal anxiety are associated with altered cognitive control in five-year-old children. *Developmental Psychobiology*, *54*(4), 441-450. https://doi: 10.1002/dev.20606

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature reviews neuroscience*, *10*(6), 434-45. https://doi: 10.1038/nrn2639.

MacKinnon, N., Kingsbury, M., Mahedy, L., Evans, J., & Colman, I. (2018). The association between prenatal stress and externalizing symptoms in childhood: Evidence from the Avon Longitudinal Study of Parents and Children. *Biological psychiatry*, *83*(2), 100-108. https://doi.org/10.1016/j.biopsych.2017.07.010

Merlot, E., Couret, D., & Otten, W. (2008). Prenatal stress, fetal imprinting and immunity. *Brain, Behavior, and Immunity*, 22(1), 42–51. https://doi.org/10.1016/j.bbi.2007.05.007

Mills-Koonce, W. R., Willoughby, M. T., Zvara, B., Barnett, M., Gustafsson, H., Cox, M. J., & Investigators, the F. L. P. K. (2015). Mothers' and Fathers' Sensitivity and Children's Cognitive Development in Low-Income, Rural Families. *J Appl Dev Psychol*, 38, 1–10. https://doi.org/10.1080/10937404.2015.1051611.

Murphy, V. E., Smith, R., Giles, W. B., & Clifton, V. L. (2006). Endocrine regulation of human fetal growth: the role of the mother, placenta, and fetus. *Endocrine Reviews*, 27(2), 141–169.

Nater, U. M., & Rohleder, N. (2009). Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology*, 34(4), 486–496. https://doi.org/10.1016/j.psyneuen.2009.01.014

Nazzari, S., Fearon, P., Rice, F., Dottori, N., Ciceri, F., Molteni, M., & Frigerio, A. (2019). Beyond the HPA-axis: exploring maternal prenatal influences on birth outcomes and stress reactivity. *Psychoneuroendocrinology*, *101*, 253-262. https://doi: 10.1016/j.psyneuen.2018.11.018.

Nazzari, S. & Frigerio, A. (2019). The programming role of maternal antenatal inflammation on infants' early neurodevelopment: A review of human studies. Special Section on "Translational and Neuroscience Studies in Affective Disorders" *Journal of Affective Disorders.* https://doi: 10.1016/j.jad.2019.10.010.

Noorlander, C. W., De Graan, P. N. E., Middeldorp, J., Van Beers, J., & Visser, G. H. A. (2006). Ontogeny of hippocampal corticosteroid receptors: effects of antenatal glucocorticoids in human and mouse. *Journal of Comparative Neurology,* 499(6), 924–932.

Nulman, I., Koren, G., Rovet, J., Barrera, M., Pulver, A., Streiner, D., & Feldman, B. (2012). Neurodevelopment of children following prenatal exposure to venlafaxine, selective serotonin reuptake inhibitors, or untreated maternal depression. *American Journal of Psychiatry*, *169*(11), 1165-1174. https://doi: 10.1176/appi.ajp.2012.11111721.

O' Connor, T. G. , Heron, J., Golding, J., Beveridge, M., & Glover, V.T.E. (2002). Maternal antenatal anxiety and children ' s behavioural/emotional problems at 4 years: Report from the Avon Longitudinal Study of Parents and Children. *The British Journal of Psychiatry,* 180, 502–508.

O'Donnell, K. J., & Meaney, M. J. (2017). Fetal origins of mental health: the developmental origins of health and disease hypothesis. *American Journal of Psychiatry*, *174*(4), 319-328. https://doi: 10.1176/appi.ajp.2016.16020138.

O'Leary, N., Jairaj, C., Molloy, E. J., McAuliffe, F. M., Nixon, E., & O'Keane, V. (2019). Antenatal depression and the impact on infant cognitive, language and motor development at six and twelve months postpartum. *Early human development*, *134*, 41-46. https://doi: 10.1016/j.earlhumdev.2019.05.021.

Osborne, S., Biaggi, A., Chua, T. E., Du Preez, A., Hazelgrove, K., Nikkheslat, N., … Pariante, C. M. (2018). Antenatal depression programs cortisol stress reactivity in offspring through increased maternal inflammation and cortisol in pregnancy: The Psychiatry Research and Motherhood – Depression (PRAM-D) Study. *Psychoneuroendocrinology.* Dec;98:211-221 https:// doi: 10.1016/j.psyneuen.2018.06.017

Owen, D., Andrews, M. H., & Matthews, S. G. (2005). Maternal adversity, glucocorticoids and programming of neuroendocrine function and behaviour. *Neuroscience & Biobehavioral Reviews,* 29(2), 209–226.

Patterson, P.H. (2002). Maternal infection: window on neuroimmune inter- actions in fetal brain development and mental illness*. Curr Opin Neurobiol,* 12, 115–118.

Patterson, P.H. (2009). Immune involvement in schizophrenia and autism: etiology, pathology and animal models. *Behav Brain Res* 204, 313–321. https://doi: 10.1016/j.bbr.2008.12.016.

Pedrabissi, L., & Santinello, M. (1989). Inventario per l'ansia di «Stato» e di «Tratto»: nuova versione italiana dello STAI Forma Y: Manuale. Firenze: Organizzazioni Speciali, 44. JOUR.

Peet, E. D., McCoy, D. C., Danaei, G., Ezzati, M., Fawzi, W., Jarvelin, M. R., ... & Fink, G. (2015). Early childhood development and schooling attainment: Longitudinal evidence from British, Finnish and Philippine birth cohorts. *PloS one*, *10*(9), e0137219. https://doi: 10.1371/journal.pone.0137219

Plamondon, A., Akbari, E., Atkinson, L., Steiner, M., Meaney, M. J., Fleming, A. S., & MAVAN research team. (2015). Spatial working memory and attention skills are predicted by maternal stress during pregnancy. *Early human development*, *91*(1), 23-29. https://doi: 10.1016/j.earlhumdev.2014.11.004.

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology,* 28(7), 916–931

Rakers, F., Rupprecht, S., Dreiling, M., Bergmeier, C., Witte, O. W., & Schwab, M. (2017). Transfer of maternal psychosocial stress to the fetus. *Neuroscience & Biobehavioral Reviews*. https://doi: 10.1016/j.neubiorev.2017.02.019

Rasmussen, J. M., Graham, A. M., Entringer, S., Gilmore, J. H., Styner, M., Fair, D. A., ... & Buss, C. (2019). Maternal Interleukin-6 concentration during pregnancy is associated with variation in frontolimbic white matter and cognitive development in early life. *NeuroImage*, *185*, 825-835. https://doi: 10.1016/j.neuroimage.2018.04.020

Raven, J. C., & Court, J. H. (1998). Raven's progressive matrices and vocabulary scales. Oxford pyschologists Press.

Rice, F., Langley, K., Woodford, C., Davey-Smith, G., & Thapar, A. (2018). Identifying the contribution of prenatal risk factors to offspring development and psychopathology: what designs to use and a critique of literature on maternal smoking and stress in pregnancy. *Development and Psychopathology,* 30(3):1107-1128. https://doi: 10.1017/S0954579418000421.

Rohleder, N., Aringer, M., & Boentert, M. (2012). Role of interleukin-6 in stress, sleep, and fatigue. *Annals of the New York Academy of Sciences*, 1261(1), 88–96. https://doi.org/10.1111/j.1749-6632.2012.06634.x

Rudolph, M. D., Graham, A. M., Feczko, E., Miranda-Dominguez, O., Rasmussen, J. M., Nardos, R., ... & Fair, D. A. (2018). Maternal IL-6 during pregnancy can be estimated from newborn

brain connectivity and predicts future working memory in offspring. *Nature neuroscience*, *21*(5), 765. https://doi: 10.1038/s41593-018-0128-y.

Segerstrom, S. C., & Miller, G. E. (2004). Psychological Stress and the Human Immune System: A Meta-Analytic Study of 30 Years of Inquiry. *Psychological Bulletin*, 130(4), 601–630.

Shenkin, S. D., Starr, J. M., & Deary, I. J. (2004). Birth weight and cognitive ability in childhood: a systematic review. *Psychological bulletin*, *130*(6), 989-1013.

Short, S.J., Lubach, G.R., Karasin, A.I., Olsen, C.W., Styner, M., Knickmeyer, R.C., Gilmore, J.H., Coe, C.L. (2010) Maternal influenza infection during preg- nancy impacts postnatal brain development in the rhesus monkey. *Biol Psychiatry*, 67, 965–973. https://doi:10.1016/j.biopsych.2009.11.026.

Skogstrand, K., Ekelund, C. K., Thorsen, P., Vogel, I., Jacobsson, B., Nørgaard-Pedersen, B., & Hougaard, D. M. (2008). Effects of blood sample handling procedures on measurable inflammatory markers in plasma, serum and dried blood spot samples. *Journal of Immunological Methods*, 336(1), 78–84. https://doi: 10.1016/j.jim.2008.04.006.

Spann, M. N., Monk, C., Scheinost, D., & Peterson, B. S. (2018). Maternal immune activation during the third trimester is associated with neonatal functional connectivity of the salience network and fetal to toddler behavior. *Journal of Neuroscience*, *38*(11), 2877-2886. https://10.1523/JNEUROSCI.2272-17.2018.

Spielberger, C. D., Gorsuch, R. L., Lushene, R. E., Vagg, P. R., & Jacobs, G. A. (1970).State-trait anxiety inventory. Palo Alto. Consulting psychologists press.

Tarabulsy, G. M., Pearson, J., Vaillancourt-Morel, M.-P., Bussières, E.-L., Madigan, S., Lemelin, J.-P., … Royer, F. (2014). Meta-analytic findings of the relation between maternal prenatal stress and anxiety and child cognitive outcome. *Journal of Developmental and Behavioral Pediatrics*, 35(1), 38–43. https://doi.org/10.1097/DBP.0000000000000003

Taylor-Colls, S., & Pasco Fearon, R. M. (2015). The Effects of Parental Behavior on Infants' Neural Processing of Emotion Expressions. *Child Development*, 86(3), 877–888. https://doi.org/10.1111/cdev.12348

Tse, A. C., Rich-Edwards, J. W., Rifas-Shiman, S. L., Gillman, M. W., & Oken, E. (2010). Association of maternal prenatal depressive symptoms with child cognition at age 3 years. *Paediatric and perinatal epidemiology*, *24*(3), 232-240. https://doi: 10.1111/j.1365-3016.2010.01113.x.

Van den Bergh, B. R., van den Heuvel, M. I., Lahti, M., Braeken, M., de Rooij, S. R., Entringer, S., ... & Schwab, M. (2017). Prenatal developmental origins of behavior and mental health: The influence of maternal stress in pregnancy. *Neuroscience & Biobehavioral Reviews*. https:// 10.1016/j.neubiorev.2017.07.003

Voegtline, K. M., Costigan, K. A., Kivlighan, K. T., Laudenslager, M. L., Henderson, J. L., & Dipietro, J. A. (2013). Concurrent levels of maternal salivary cortisol are unrelated to self-reported psychological measures in low-risk pregnant women. *Archives of Women's Mental Health*, 16(2), 101–108. https://doi: 10.1007/s00737-012-0321-z

Wium-Andersen, M. K., & Nielsen, S. F. (2013). Elevated C-Reactive Protein Levels, Psychological Distress, and Depression in 73131 Individuals. *JAMA Psychiatry*, 70(2), 176–184. https://doi: 10.1001/2013.jamapsychiatry.102.

Wu, T. L., Tsao, K. C., Chang, C. P. Y., Li, C. N., Sun, C. F., & Wu, J. T. (2002). Development of ELISA on microplate for serum C-reactive protein and establishment of age-dependent normal reference range. *Clinica Chimica Acta*, *322*(1-2), 163-168.

TABLES

Table 1 – Means, Standard Deviations (SD) and Ranges for all study variables

Study Variable	Mean	SD	Range
Maternal cortisol (µg/dl)			
Waking	0.38	0.13	0.13-0.83
Waking +30’	0.50	0.15	0.10-0.91
Bedtime	0.18	0.06	0.01-0.41
AUCg	263.92	58.90	83.76-442.71
Maternal sAA (U/ml)			
Waking	69.89	64.68	3.00-463.84
Waking +30’	48.33	38.65	2.80-190.10
Bedtime	97.90	80.39	3.28-562.71
AUCg	3612.88	690.74	1777.74-5394.20
Maternal CRP (ng/ml)	3749.61	2714.57	480.04-11179.80
Maternal IL-6 (pg/ml)	1.69	1.03	0.48-6.47
Maternal depression (EPDS)	5.34	4.34	0-19
Maternal anxiety (STAI-S)	34.95	8.63	20-58
Infant cognitive composite score	102.36	10.81	75-125

Note: AUCg, Area Under the Curve with respect to the ground; sAA, salivary Alpha Amylase; CRP, C-Reactive Protein; IL-6, Interleukin-6; EPDS, Edinburgh Postnatal Depression Scale score; STAI-S, State-Trait Anxiety Inventory state score

Table 2 – Bivariate correlations among study variables

	1	2	3	4	5	6
1. Prenatal EPDS						
2. Prenatal STAI-S	**.60****					
3. Prenatal Cortisol AUCg	-.14	.04				
4. Prenatal sAA AUCg	.09	.20	**.23***			
5. Prenatal IL-6	**.**19	.20	.11	.18		
6. Prenatal CRP	.06	.13	.09	.01	**.30****	
7. Infant Cognitive composite score	-.03	-.09	**-.26****	-.08	-.07	-.17

Note: EPDS, Edinburgh Postnatal Depression scale; STAI-S, state subscale of the State-Trait Anxiety Inventory; AUCg, area under the curve with respect to the ground; sAA, salivary Alpha Amylase; IL-6, Interleukine-6; CRP, C-Reactive Protein.

* p<.05; **p<.01, p>.05 was considered non-significant.

Table 3 – Hierarchical linear regression analyses predicting 3-month-olds' cognitive development

	Cognitive composite scores							
	Cortisol AUCg		sAA AUCg		IL-6		CRP	
	β	p	β	p	β	p	β	p
Step 1:								
Gender	.14	.17	.12	.20	.09	.40	.08	.45
Gestational Age	.19	.05	**.20**	**.04**	.18	.09	.18	.09
Maternal IQ	.18	.07	.18	.07	.12	.30	.09	.41
Maternal EA	.16	.11	.16	.10	**.23**	**.04**	**.23**	**.04**
Postnatal EPDS	.04	.67	.11	.35	.03	.88	.04	.73
ΔR^2 for step 1	**.14**	**.02**	**.14**	**.02**	.13	.05	.13	.05
F_{model}	**2.89**	**.02**	**2.96**	**.02**	2.38	.05	2.37	.05
Step 2:								
Prenatal EPDS	-.15	.21	-.13	.27	-.16	.27	-.14	.28
ΔR^2 for step 2	.01	.21	.01	.27	.01	.24	.01	.28
F_{model}	**2.68**	**.02**	**2.68**	**.02**	2.25	.05	2.17	.05
Step 3:								
Prenatal biomarkers	**-.37**	**<.001**	-.09	.38	-.01	.95	**-.22**	**.04**
ΔR^2 for step 3	**.12**	**<.001**	.01	.38	.00	.95	**.04**	**.04**
F_{model}	**4.79**	**<.001**	**2.41**	**.03**	1.88	.08	**2.54**	**.02**

Note: AUCg, Area Under the Curve with respect to the ground; sAA, salivary Alpha Amylase; CRP, C-Reactive Protein; IL-6, Interleukin-6; EPDS, Edinburgh Postnatal Depression Scale score; EA, Emotional Availability.

AVAILABILITY OF DATA STATEMENT: The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPLEMENTARY MATERIALS – “Neuroendocrine and immune markers of maternal stress during pregnancy and infant cognitive development”

Supplementary Table 1 – Hierarchical linear regression analyses predicting 3-month-olds’ cognitive development

	Cognitive composite scores							
	Cortisol AUCg		sAA AUCg		IL-6		CRP	
	β	p	β	p	β	p	β	p
Step 1:								
Gender	.12	.22	.11	.26	.07	.49	.07	.54
Gestational Age	.19	.06	**.19**	**.05**	.17	.10	.17	.10
Maternal IQ	.19	.06	.18	.06	.12	.30	.10	.38
Maternal EA	.14	.15	.15	.13	**.21**	**.05**	**.22**	**.05**
Postnatal STAI	.06	.52	.06	.53	.08	.44	.08	.45
ΔR^2 for step 1	**.13**	**.02**	**.13**	**.02**	**.12**	**.05**	.12	.06
F_{model}	**2.76**	**.02**	**2.84**	**.02**	**2.27**	**.05**	2.25	.06
Step 2:								
Prenatal STAI	-.14	.20	-.13	.22	-.11	.37	-.11	.38
ΔR^2 for step 2	.01	.20	.01	.22	.01	.37	.01	.38
F_{model}	**2.59**	**.02**	**2.64**	**.02**	2.02	.07	2.00	.07
Step 3:								
Prenatal biomarkers	**-.32**	**.001**	-.06	.57	-.04	.73	**-.20**	**.05**
ΔR^2 for step 3	**.09**	**<.001**	.00	.57	.00	.73	**.04**	**.05**
F_{model}	**4.02**	**.001**	**2.29**	**.03**	1.73	.11	**2.30**	**.03**

Note: AUCg, Area Under the Curve with respect to the ground; sAA, salivary Alpha Amylase; CRP, C-Reactive Protein; IL-6, Interleukin-6; STAI, State-Trait Anxiety Inventory state anxiety subscale score; EA, Emotional Availability.