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Male fetus susceptibility to maternal inflammation: C-reactive protein and brain development

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Original Article

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Abstract

Background. Maternal inflammation in early pregnancy has been identified epidemiologically as a prenatal pathogenic factor for the offspring's later mental illness. Early newborn manifestations of the effects of maternal inflammation on human fetal brain development are largely unknown.

Methods. Maternal infection, depression, obesity, and other factors associated with inflammation were assessed at 16 weeks gestation, along with maternal C-reactive protein (CRP), cytokines, and serum choline. Cerebral inhibition was assessed by inhibitory P50 sensory gating at 1 month of age, and infant behavior was assessed by maternal ratings at 3 months of age.

Results. Maternal CRP diminished the development of cerebral inhibition in newborn males but paradoxically increased inhibition in females. Similar sex-dependent effects were seen in mothers' assessment of their infant's self-regulatory behaviors at 3 months of age. Higher maternal choline levels partly mitigated the effect of CRP in male offspring.

Conclusions. The male fetal-placental unit appears to be more sensitive to maternal inflammation than females. Effects are particularly marked on cerebral inhibition. Deficits in cerebral inhibition 1 month after birth, similar to those observed in several mental illnesses, including schizophrenia, indicate fetal developmental pathways that may lead to later mental illness. Deficits in early infant behavior follow. Early intervention before birth, including prenatal vitamins, folate, and choline supplements, may help prevent fetal development of pathophysiological deficits that can have life-long consequences for mental health.

Introduction

Retrospective epidemiological studies established maternal infection during the early second trimester as a risk factor for schizophrenia (Brown & Derkits, 2010; Clarke, Tanskanen, Huttunen, Whittaker, & Cannon, 2009). Animal models identify maternal immune activation (MIA) with cytokine induction as a pathophysiological mechanism (Wu et al., 2015). Maternal inflammation, often in response to respiratory and genitourinary infections in the early second trimester, decreases the development of inhibitory sensory gating and increases impulsivity in the offspring (Freedman et al., 2019; Ghassabian et al., 2018; Melbye, Hvidsten, Holm, Nordbø, & Brox, 2004). Pathogenic effects of maternal cytokines on both the placenta and the brain have been proposed (Brown & Meyer, 2018). In addition to infection, maternal inflammation has been associated with obesity, maternal depression and anxiety, and environmental pollution (Madan et al., 2009; Osborne & Monk, 2013; van den Hooven et al., 2012). Elevation in plasma C-reactive protein (CRP) at 16 weeks gestation and interleukin-6 (IL-6) later in pregnancy in women whose offspring develop schizophrenia adds molecular evidence for the pathogenic role of inflammation (Canetta et al., 2014; Goldstein et al., 2014). These epidemiological findings are now well-accepted, but limited information exists about which specific aspects of human fetal brain development are altered by maternal inflammation to produce mental illness in later life (Graham et al., 2018; Spann, Monk, Scheinost, & Peterson, 2018). The first pathophysiological signs of schizophrenia, apparent as early as birth, have been proposed as indicators of aberrant fetal brain development (Erlenmeyer-Kimling & Cornblatt, 1987; Walker, Savoie, & Davis, 1994). RDoC provides a framework that can be applied prospectively to study the earliest development origins of schizophrenia by examining pathophysiological components of the illness that appear before diagnostic symptoms are apparent (Ross & Freedman, 2015).

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This study assessed newborn cerebral inhibitory physiology as a primary outcome of fetal brain development using an auditory sensory gating measure, a biomarker of schizophrenia in adults that first appears in newborns (Kisley, Polk, Ross, Levisohn, and Freedman, 2003). Development of inhibitory neurons was characterized by sensory gating of the P50 cerebral evoked response in a paired-auditory stimulus paradigm (S1, S2). P50 amplitude is normally decremented in response to S2, because of inhibitory mechanisms activated by the response to S1. This inhibition is impaired in persons with schizophrenia (Adler et al., 1982). P50 sensory gating is a physiological measure in the RDoC Cognition Domain, Perception Construct, Auditory Sub-Construct. P50 sensory gating has been used in adults with schizophrenia to detect familial or genetic risk (Freedman et al., 1997; Hall, Taylor, Salisbury, & Levy, 2011; Quednow et al., 2012). Abnormal P50 gating also occurs in autism spectrum disorder (Olincy, Blakeley-Smith, Johnson, Kem, & Freedman, 2016; Orekhova et al., 2008). P50 inhibition can be recorded as soon as 1 month after birth (Kisley et al., 2003). Diminished newborn P50 inhibition predicts early childhood problems in self-regulation, attention, and social function (Hutchison et al., 2007; Ross et al., 2016). These early childhood behaviors are recognized as early developmental symptoms in children who later develop psychosis (Pine & Fox, 2015; Rossi, Pollice, Daneluzzo, Marinangeli, & Stratt, 2000; Rutter, Kim-Cohen, & Maughan, 2006). Newborn P50 inhibitory deficits are increased if either parent has schizophrenia, a finding replicated by another group (Hunter, Kisley, McCarthy, Freedman, & Ross, 2011; Smith, Crawford, Thomas, & Reid, 2018).

Increased maternal choline promotes the development of newborn P50 inhibitory gating, and therefore, we also assessed the possible interaction of the maternal inflammatory response with choline in this study (Ross et al., 2013).

Participants and methods

Maternal assessment and recruitment

Women were enrolled from a public safety-net prenatal clinic at 14–16 weeks gestation from July 2013 until July 2016. Gestational age was timed from the last menstrual period and by ultrasound. Exclusions were fetal anomaly and major maternal medical morbidity. The Colorado Multiple Institution Review Board approved the study; all participants gave informed consent. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Women were asked to participate in a prospective study of stress in pregnancy on their child's development.

Psychiatric diagnoses were made using the Structured Clinical Interview for DSM-IV Axis I Disorders with DSM-5 criteria. Self-ratings on Center for Epidemiological Studies of Depression (CESD), State-Trait Anxiety Inventory-State Version (STAI-S), and the Perceived Stress Scale (PSS) were acquired. Maternal sociodemographics and health, including infections, substance use, BMI, and prenatal vitamin use, were assessed.

The medical record for all prenatal care was reviewed for infection. A mother's self-report of infection was considered significant if it was entered as a problem in the medical record. Treatment was provided for all reported genitourinary infections. Most respiratory infections were viral and were therefore not treated. In addition, mothers had an in-person review of systems for symptoms of infection at 16 weeks gestation by research personnel. The correlation between symptoms rated by the mother as moderate to severe on a custom-made scale from little or mild (1–3) to moderate (4–5) to severe (6–9) in the interview and problems in the medical record is $r_s = 0.96$, $p < 0.001$ (Freedman et al., 2019). Mothers reported the use of alcohol, cannabis, and nicotine at 16 weeks gestation in the same interview and at 6-week intervals through the first 6 weeks postpartum (Hoffman et al., 2019a, 2019b).

Labor, delivery, and neonatal parameters were recorded from the medical record. Investigators were blinded to maternal choline levels during assessments.

Choline measurements

Maternal plasma was assayed for choline and its metabolite betaine at 16 weeks gestation by the Colorado Translational Research Center Metabolomics Laboratory using mass spectroscopy (online Supplementary material). Blood samples were obtained at least 2 h after breakfast. Plasma was quickly separated by refrigerated centrifugation to prevent platelet phosphatidylcholine release.

Mothers received information on diets higher in choline, but dietary intake was not estimated because of the low relationship of self-reported intake to maternal choline levels in pregnant women, $r = 0.2$ (Wu, Dyer, King, Richardson, & Innis, 2012). The placental choline transporter CLT1 produces amniotic fluid levels approximately twice maternal plasma levels (Baumgartner et al., 2015; Ilcol, Uncu, & Ulus, 2002). Uptake is proportional to maternal plasma concentration, which suggests that higher peak levels may be important determinants of amniotic fluid levels (Iwao et al., 2016). Maternal levels obtained in non-fasting conditions, as in the present study, can be elevated, but only after high-choline meals that exceed the recommended daily intake (Abratte et al., 2009; Holm, Ueland, Kvalheim, & Lien, 2003; Zeisel, Growden, Wurtman, Magil, & Logue, 1980). Only choline activates α7-nicotinic receptors (Alkondon, Pereira, Cortes, Maelicke, & Albuquerque, 1997). We found no effects of its metabolite betaine.

Maternal CRP and cytokines

Plasma CRP at 16 weeks gestation was assayed by the Beckman–Coulter high sensitivity assay at the Colorado Translational Research Center, Colorado Children's Hospital. Tumor necrosis factor-α (TNFα), IL-6 and IL-8 were assayed by R&D Systems high sensitivity assays at the CTRC Core Laboratory, Children's Hospital of Colorado. IL-6 values above the detectable limit (1.9 pg/ml) were found for nine of 91 samples from women without infection and 17 of 61 women with infection (Fisher's exact test $p = 0.007$). TNF levels above detectable limits were observed in 81 of 91 women without infection and 60 of 61 women with infection (1.6 pg/ml). Levels below detectable limits were set to zero.

Neonatal physiological recording of cerebral inhibition

Newborns were studied 1 month (44 weeks after the last menstrual period) after birth adjusted for gestational age. Vertex electroencephalogram, electro-oculogram, submental electromyogram, and respiration were continuously recorded while infants napped (Hunter et al., 2011; Kisley et al., 2003). Recording of

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the cerebral auditory evoked potential P50, a positive EEG wave 50 ms post stimulus, occurred in the second active sleep episode, the precursor of REM sleep, identified by low-voltage desynchronized vertex activity with the absence of K-complexes, change in respiration, and large eye movements with submental atonia (Anders, Emde, & Parmelee, 1971). The second active sleep episode was reached approximately 45 min after sleep onset. In adults, P50 inhibition in REM and waking are equivalent (Griffith & Freedman, 1995).

Two identical auditory stimuli are delivered 500 ms apart to elicit $P50_{S1}$ and $P50_{S2}$. P50 inhibition is often assessed as amplitude ratios $P50_{S2}/P50_{S1}$ or $(P50_{S1}–P50_{S2})/P50_{S1}$ (Adler et al., 1982). However, the skew inherent in ratios limits their power for correlation with risk factors. $P50_{S2}$ amplitude, covaried for $P50_{S1}$, which is normally distributed, has therefore also been used (Smith, Boutros, & Schwarzkopf, 1994). Two large NIMH genetic collaborations that included P50 sensory gating in their phenotypes [eight-site Collaboration on the Genetics of Schizophrenia (COGS) and five-site Bipolar-Schizophrenia Network on Intermediate Phenotypes (B-SNIP)] found effect sizes $d' = 0.28$ and $d' = 0.22$, respectively, for $P50_{S2}$ amplitude in schizophrenia, compared to community controls (Clementz et al., 2016; Olincy et al., 2010). A recent study using magnetoencephalography also reported that the sensory gating defect in schizophrenia is robust to the measurement method (Schubring, Tzvetan, Miller, & Rockstroh, 2018). Regardless of the method used, higher $P50_{S2}$ amplitudes indicate decreased inhibition. The assumption is that $P50_{S1}$ variance is small, compared to $P50_{S2}$ variance. In 151 newborns, effect sizes for $P50_{S1}$ differences between newborns whose mothers had no known risk *v.* women with depression or schizophrenia ranged from 0 to 0.16. Effect sizes for the decrease in $P50_{S2}$ amplitude were 0.21–0.50 (Hunter et al., 2011). Intraclass correlation between two newborn recordings 1 week apart is $r_{ICC} = 0.84$. Other technical aspects of recordings have been published (Hunter, Corral, Ponicsan, & Ross, 2008; 2015).

Childhood behavioral assessments

Parents completed the Infant Behavior Questionnaire-Revised Short Form (IBQ-R) when the infant was 3 months of age (Gartstein & Rothbart, 2003; Putnam, Helbig, Gartstein, Rothbart, & Leerkes, 2014). The Parental Distress Subscale of the Parenting Stress Index was also completed as a possible covariate for parental bias (Abidin & FL, 2012). The 91-item IBQ-R Short Form, commonly used to study behavior in children at this age, rates 14 aspects of child behavior, which the IBQ-R developers clustered into three indices by factor analysis: Surgency summarizes the child's level of activity and positive affect; Negativity summarizes fearfulness and anxiety; and Regulation summarizes duration of attention, responsiveness to parents, and enjoyment of quiet play. Two components of Regulation are also in Surgency, smiling and soothability, and the two indices are highly correlated in the present sample ($r = 0.51$, $p < 0.001$). Covariation with Surgency isolates elements of Regulation that are more specific to the early development of attention and less attributable to the child's general psychomotor activation. A similar covariance between Regulation and Surgency (0.80) has been documented by another group, who have also proposed revisions to the factor structure (Bosquet-Enlow, White, Hails, Cabrera, & Wright, 2016). Lower IBQ-R Regulation at 1 year of age is associated with decreased reading readiness at age 4 years and decreased conscientiousness, organization, and increased distractibility at age 9 years of age (Putnam et al., 2014; Slobodskaya & Kozlova, 2016).

Statistical analyses

Neonatal $P50_{S2}$ amplitude and childhood IBQ-R Regulation were the two principal outcomes, based on previous work that found P50 inhibition was a biomarker of both infection and choline's effects and that regulatory behaviors were the most affected outcome (Freedman et al., 2019; Ross et al., 2016). Kolmogorov–Smirnoff tests for each outcome did not find a significant deviation from normal distributions. General Linear Models and multiple regression analyzed child sex as a fixed effect and CRP and other cytokine levels and choline levels as a continuous effect. For $P50_{S2}$ amplitude, maternal tobacco smoking, cannabis, and alcohol use were examined as covariates because of previously established effects on P50 inhibition (Hoffman et al., 2019a, 2019b; Hunter et al., 2011). For IBQ-R analyses, maternal education was a covariate because of its correlation with both CRP levels ($r = –0.169$, $p = 0.038$) and IBQ-R Regulation ($r = 0.196$, $p = 0.021$). In addition, maternal 3 months postpartum CESD, STAI-S, PSS, and PSI were examined as covariates, because of possible bias in ratings from maternal mood and stress. In a previous study, the effect size of choline on P50 inhibition was Cohen's $d' = 0.7$ (Ross et al., 2013). We expected 20% of the women would have adequate choline levels and 30% attrition (Hoffman et al., 2019a; 2019b; Wu et al., 2012). Therefore, we enrolled 200 women to have power $1–\beta > 0.95$, $\alpha = 0.05$, 1-tail to observe an overall choline effect.

Results

Mothers' inflammatory response during early gestation

Of 316 mothers screened, 201 were enrolled by 16 weeks gestation, as dated from the last menstrual period and verified by ultrasound measurements. Of these, 162 brought their newborns to the 1-month postnatal assessment, where the auditory evoked potentials were obtained as previously reported (Freedman et al., 2019). CRP levels were assessed in plasma samples obtained at 16 weeks gestation from 150 of the women (online Supplementary Fig. S1). When the infants reached 3 months of age, 127 women brought them in for a behavioral assessment. Attrition during the study after enrollment generally reflected mothers who moved from the pre-birth residence to their mothers' homes to care for their infants and were then lost to follow-up. The significant differences between male and female babies were only in greater male birth weights and head circumferences (online Supplementary Table S1).

CRP levels correlated with maternal CESD self-ratings of depression, pre-pregnancy BMI, severity of gestational infection, and maternal education (Table 1). There was no correlation with infant sex. More educated women were older ($r = 0.324$, $p < 0.001$) and had lower self-ratings of depression ($r = –0.190$, $p = 0.015$) and lower BMI ($r = –0.283$, $p < 0.001$), which has been found in other populations (Bui & Miller, 2018). CESD ratings correlated with both infection ($r = 0.264$, $p = 0.001$) and BMI ($r = 0.210$, $p = 0.007$). Multiple regression analysis found significant effects for CRP of infection severity [$\beta = 0.202$ (95% CI 0.047–0.356) $p = 0.011$] and BMI [$\beta = 0.329$ (95% CI 0.176–0.482) $p < 0.001$], but not depression or maternal education (online Supplementary Table S2). If the ratings were dichotomized to

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Table 1. Maternal factors associated with CRP elevation at 16 weeks gestation

Parameters	Pearson *r*	*p*
Child sex	−0.129	0.12
Pre-pregnancy BMI	0.358	<0.001
Infection severity rating 16 weeks	0.245	0.002
Clinician-reported moderately severe infection 16 weeks	0.235	0.004
CESD-R 16 weeks	0.175	0.03
Maternal educations years	−0.169	0.04
Maternal age years	0.051	0.53
Bipolar disorder	−0.049	0.55
Depressive disorder	0.078	0.34
Anxiety disorder	−0.103	0.21
Schizophrenia	0.045	0.58
Nicotine use	0.058	0.48
Alcohol use	0.036	0.66
Cannabis use	0.094	0.26
CESD > 15	0.094	0.26
Antidepressant use	0.107	0.19
STAI 16 weeks	0.018	0.82
PSS 16 weeks	0.102	0.22
Antibiotic use	0.022	0.79
Obesity BMI⩾30	0.030	0.71
Other maternal cytokines		
IL-6 16 weeks	0.543	<0.001
IL-8 16 weeks	0.198	0.02
TNF-α 16 weeks	0.186	0.02

identify mothers likely to come to clinical attention as depressed (CESD > 15), obese (BMI > 30), or as having a significant infection (severity>5), then only infection was a significant factor for CRP elevation [$\beta = 0.237$ (95% CI 0.076–0.398) $p = 0.004$].

CRP levels were not associated with either complications of labor and delivery, such as preterm birth, gestational diabetes, or chorioamnionitis, or neonatal outcomes, such as lower APGAR scores or small size for gestational age infants (online Supplementary Table S3). CRP levels were correlated with other maternal cytokines, specifically IL-6, IL-8, and TNF-α (Table 1).

Newborn expression of cerebral inhibition

A paired-stimulus auditory paradigm (S1, S2) delivered during active sleep was used to assess the newborn's development of cerebral inhibition, measured as the decrease in amplitude of $P50_{S2}$, relative to $P50_{S1}$ (Fig. 1). There were no differences in $P50_{S1}$ or in $P50_{S2}$ amplitude between male and female newborns (Table 1).

Maternal CRP levels had a sex-dependent effect on $P50_{S2}$ amplitude (Wald $\chi^2_{df1} = 6.522$, $p = 0.011$; online Supplementary Table S4); $P50_{S1}$ was unaffected. CRP levels correlated with increased $P50_{S2}$ amplitude in males [$\beta = 0.205$ (95% CI 0.023–0.387) $p = 0.03$], but decreased $P50_{S2}$ amplitude in females [$\beta = -0.214$ (95% CI −0.390 to −0.038) $p = 0.016$; Fig. 1]. Tobacco smoking, marijuana, and alcohol use during early pregnancy did not affect the significance of the effects of CRP on $P50_{S2}$ amplitude (online Supplementary Table S4).

Infant behavior at 3 months of age

Maternal ratings of their infants' behavior showed no differences between male and female infants on the three principal IBQ-R indices – Surgency, Regulation, and Negativity (online Supplementary Table S1). Multivariate analysis of effects on CRP on the three IBQ-R indices found that maternal CRP increased 3-month IBQ-R Surgency regardless of the sex of the infant [$F_{df1} = 4.645$, $p = 0.033$; $\beta = 0.207$ (95% CI 0.036–0.378); online Supplementary Table S5]. The two most affected components of this scale were Vocalizations (Wald $\chi^2_{df1} = 6.299$, $\beta = 0.195$, $p = 0.012$); and High Intensity Pleasure (Wald $\chi^2_{df1} = 7.478$, $\beta = 0.202$, $p = 0.006$).

The effects of CRP on IBQ-R Regulation were sex-dependent (CRP × sex: $F_{df1} = 4.291$, $p = 0.041$; Fig. 3; online Supplementary Table S5). There were no significant effects of CRP on IBQ-R Negativity. In males, CRP decreased Regulation [$\beta = -0.209$ (95% CI −0.429 to −0.001), $p = 0.049$], but there was no significant effect in females ($\beta = 0.062$, $p = 0.6$). The two most affected IBQ-R components were Soothability (CRP × sex: Wald $\chi^2_{df1} = 4.842$, $p = 0.042$; in males $\beta = -0.388$, $p = 0.005$) and Cuddliness (CRP × sex: Wald $\chi^2_{df1} = 6.043$, $p = 0.014$; in males $\beta = -0.245$, $p = 0.044$).

Multiple regression found that newborn $P50_{S2}$ amplitude was related to 3-month-old infant IBQ-R Regulation, also in a sex-dependent manner ($P50_{S2}$ × sex: Wald $\chi^2_{df1} = 7.052$, $p = 0.008$; Fig. 2, online Supplementary Table S6).

IL-6 levels correlated with infant IBQ-R Negativity across both sexes ($r = 0.229$, $p = 0.009$).

Interaction of maternal choline levels and prenatal vitamins with the maternal inflammatory response

Maternal choline levels at 16 weeks gestation did not directly affect CRP levels, but maternal choline levels decreased the effect of CRP levels on $P50_{S2}$ amplitude. Multiple regression found effects of maternal choline for all newborns [$\beta = -0.121$ (95% CI −0.227 to −0.01); Wald $\chi^2_{df1} = 2.017$, $p = 0.029$] and sex-specific effects also (Wald $\chi^2_{df1} = 7.888$, $p = 0.019$; Fig. 3, online Supplementary Table S7). In males, choline significantly diminished $P50_{S2}$ amplitude [$\beta = -0.216$ (95% CI −0.297 to −0.035) $P = 0.019$]. In females, the effect of choline was small and not significant ($\beta = 0.010$, $p = 0.9$). To demonstrate the three-way interaction of choline with CRP and child sex, choline levels were dichotomized based on the mean value in the sample (6.4 μM). For males whose mothers had choline levels >6.4 μM, the effect of CRP on $P50_{S2}$ amplitude was considerably diminished, until CRP levels rose above 15 mg/ml (Fig. 3).

Prenatal vitamins with folic acid decreased $P50_{S2}$ amplitude in both sexes, but the effects did not reach significance (in males, $\beta = -0.168$, $p = 0.07$; in females, $\beta = -0.148$, $p = 0.09$).

Multiple regression found a significant interaction of child sex and maternal CRP and choline levels for IBQ-R Regulation (Wald $\chi^2_{df1} = 7.663$, $p = 0.022$; online Supplementary Table S8). For males whose mothers had choline levels below 6.4 μM, the effect of CRP on Regulation was $\beta = 0.352$ (95% CI 0.044–0.660), $p = 0.028$. If the mother had choline levels above 6.4 μM, the effect of CRP levels became non-significant ($\beta = 0.093$, $p = 0.6$; Fig. 3). The effect of CRP on Regulation was non-significant for females

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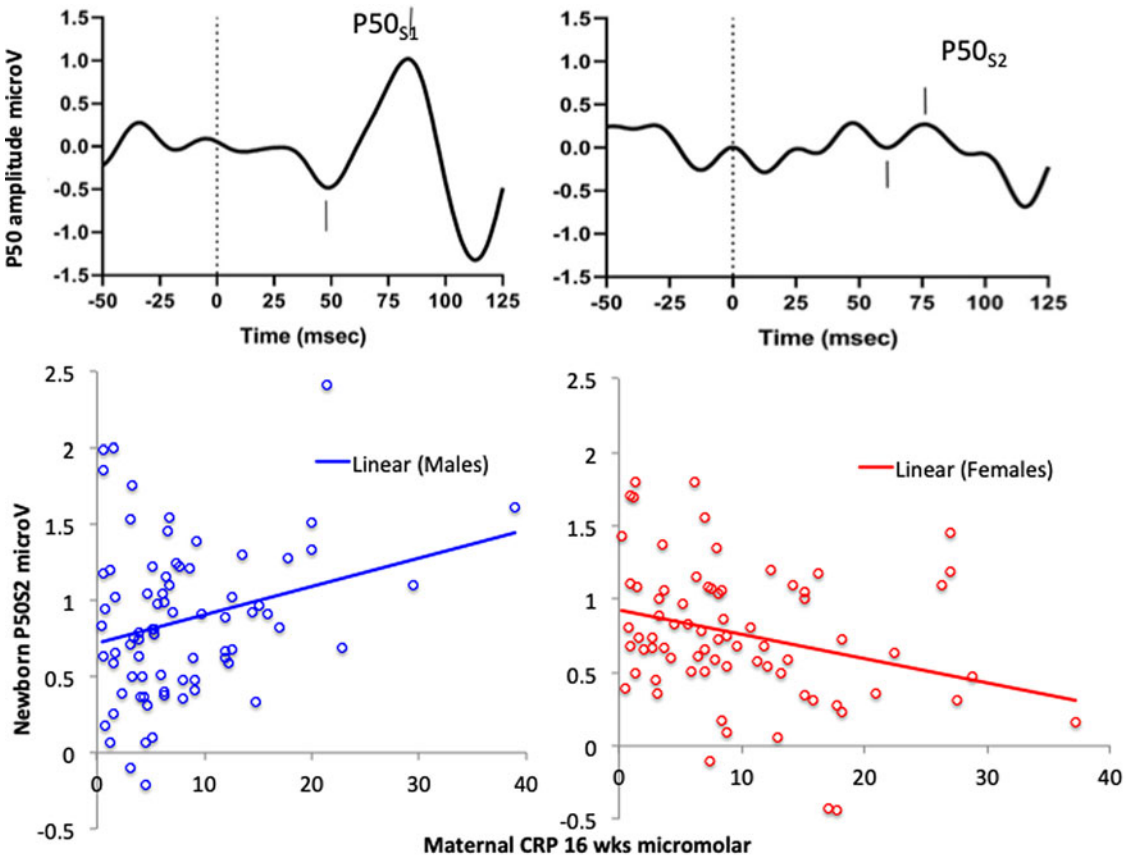


Fig. 1. Top: Examples of $P50_{S1}$ and $P50_{S2}$ auditory evoked responses recorded 1 month after birth in a female newborn. P50 amplitude is measured from the most positive peak relative to the preceding negativity. The mother's 16-week gestation CRP was 15.3 mg/ml and her choline was 5.87 μM. $P50_{S1}$ was 1.50 μV and $P50_{S2}$ was 0.27 μV. Bottom: Effects of CRP on $P50_{S2}$ for male and female infants.

regardless of maternal choline level. Prenatal vitamins had no significant effect on IBQ-R Regulation for either sex.

Comment

Increased maternal CRP levels in early gestation were associated with decreased development of P50 cerebral inhibition in males but not females. The decrease in P50 sensory inhibitory gating was further reflected in decreased infant Regulation behaviors rated on the IBQ-R at 3 months of age. CRP levels were increased by maternal infections, obesity, and depression, with infection and BMI being predominant (Freedman et al., 2019). Unlike inhibitory gating and Regulation, increases in both infant Surgency, also related to elevated CRP, and infant Negativity, related to elevated IL-6, were observed in both sexes. The sex-specific effects on inhibition in male fetuses suggest that there may be specific effects of maternal inflammation on the development of inhibitory interneurons, particularly since their development in this study appears to be facilitated in females by the inflammatory response. Other aspects of early behavior, Surgency and Negativity, are similarly affected in both sexes, however, suggesting that there may be both sex-dependent and sex-independent mechanisms affecting fetal brain development. Effects on both the placenta's integrity and neuronal development have been investigated in the maternal immune response (Brown & Meyer, 2018).

In animal models, cerebral interneurons are sensitive to maternal inflammation and hypoxia (Lacaille et al., 2019). Parvalbumin neurons of the hippocampus are specifically affected, as well as somatostatin-positive neurons (Canetta et al., 2016; Vasistha et al., 2019). A pathophysiological mechanism involving the astroglia response has been proposed (Sobue et al., 2018). Male mice have a greater reduction in hippocampal volume and more chronic macrophage infiltration than females after MIA (Dada et al., 2014). Male mice are also more sensitive to other insults, such as prenatal radiation, with greater loss of hippocampal pyramidal and interneurons (Ganapathi & Manda, 2017). We did not find a report of an animal model of inflammation that enhanced female brain development. Moreover, female mice are more sensitive to the apoptotic effects of prenatal dexamethasone (Zuloaga et al., 2012), and effects on NMDA receptors in the hippocampus after prenatal glucocorticoids are more marked in females than males (Owen, Setiawan, Li, McCabe, & Matthews, 2004). A second hit has also been proposed as a pathophysiological mechanism. Animals exposed to MIA during gestation are more likely to show deficits after pubertal stressors. This combination reduced the expression of parvalbumin inhibitory interneurons, but the analyses were performed only in male mice (Giovanoli et al., 2013). Differences in gene expression in placental trophoblasts have also been proposed as a mechanism of female resilience. The X-linked gene *OGT* is expressed at greater levels in females; it is responsible for protein glycosolation, including glycosolation of H3K27me3, which regulates repressive epigenetic modifications (Nugent, O'Donnell, Epperson, & Bale, 2018).

In humans, the retrospective epidemiological data are not as clear. Maternal CRP levels at 16 weeks gestation are associated with increased risk of schizophrenia and autism spectrum

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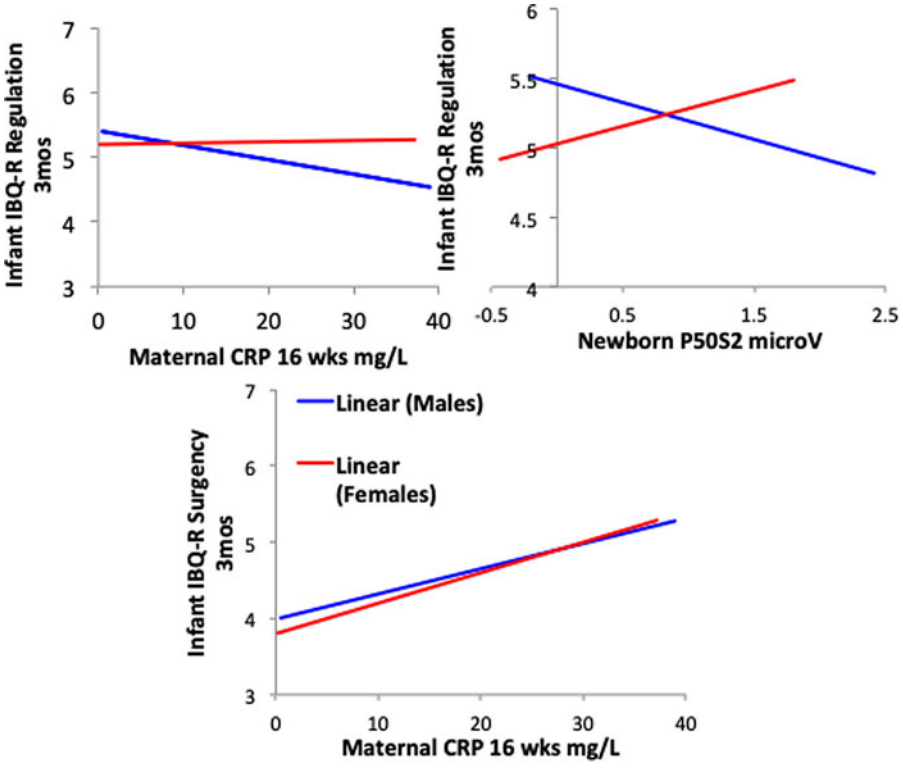


Fig. 2. Top left: Effects of maternal CRP on infant IBQ-R Regulation. Top right: Relation of newborn $P50_{S2}$ amplitude to infant IBQ-R Regulation. Bottom: Effects of maternal CRP on infant IBQ-R Surgency.

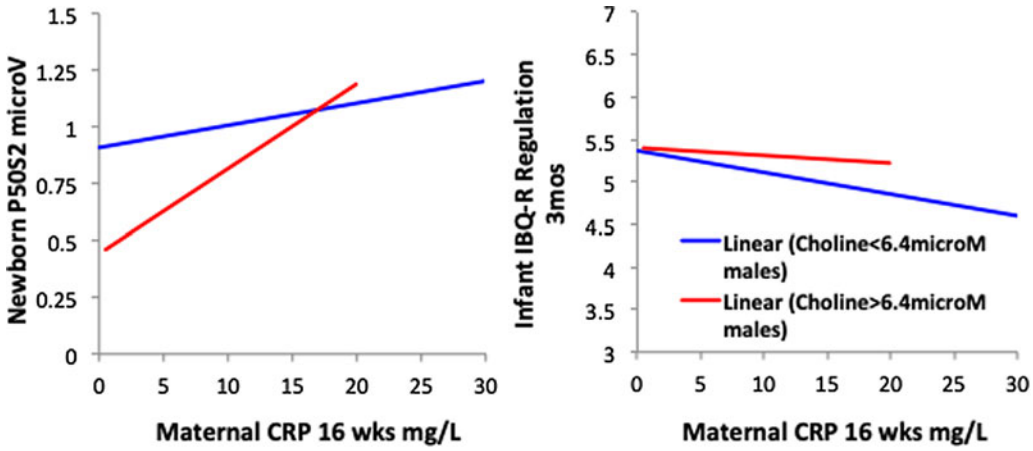


Fig. 3. Effects of maternal choline levels and maternal CRP on male newborn $P50_{S2}$ (left) and IBQ-R Regulation (right).

disorder in a Finnish cohort (Brown et al., 2014; Canetta et al., 2014), but decreased autism spectrum disorder in a California cohort (Zerbo et al., 2016). In a Dutch cohort, higher maternal CRP levels at 13 weeks gestation were related to autism traits in the general population (Koks et al., 2016). There were no sex-specific effects in any of these cohorts, despite the preponderance of schizophrenia and autism disorders in males. In a New England cohort, however, mothers of males with schizophrenia had higher IL-6 levels in the early third trimester (Goldstein et al., 2014). The double prenatal-adolescent hit is also apparently pathogenic in humans. In a Danish cohort, females had increased risk for schizophrenia after prenatal exposure to infection compared to males, but additional pubertal trauma resulted in males having a markedly increased risk (Debost et al., 2017). Like animal models, human females are also more sensitive to the effects of corticosteroids released naturally when mothers become depressed (Kim et al., 2017). Thus, there may be a second pathway by which female fetuses are affected because the mother's infection is often accompanied by depression (Freedman et al., 2019).

CRP does not generally cross from the maternal circulation to directly affect the fetus (Malek et al., 2006). A possible mechanism of the effects of MIA is cytokine-mediated macrophage attack of the placenta, which the mother's immune system may treat as a foreign body. Placental cytokines are increased in the placenta in animal models of stress during pregnancy, specifically in males (Bronson & Bale, 2014). In humans, elevated CRP in early gestation is associated with chronic placental villitis (Ernst et al., 2013). Maternal CRP is deposited in the human placenta during pregnancy, where increased levels are associated with

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chorioamnionitis, pre-eclampsia, and preterm delivery (Kim et al., 2015). Genes expressed in the placenta are associated with schizophrenia in the subset of patients who had prenatal complications, including significant infection. The expression of these genes in the placenta is specifically upregulated in males compared to females (Ursini et al., 2018). MIA of umbilical vein macrophages is also greater in males than in females (Kim-Fine et al., 2012).

This maternal immune attack on the placenta, although not directly transmitted to the fetus, triggers a reaction in the fetus itself that directly affects fetal brain development, including interneuron migration specifically (Oskvig, Elkahloun, Johnson, Phillips, & Herkenham, 2012). These interneurons are responsible for inhibition of the cerebral auditory evoked response (Miller & Freedman, 1995). The timing of measurement of maternal CRP levels, early in pregnancy *v.* closer to term, is critical for assessing pathogenic *v.* normal effects. Cerebral interneurons are in a critical stage of development at 16 weeks gestation, when we found effects in this study and when effects are also seen on the later risk for schizophrenia (Bayatti et al., 2008; Canetta et al., 2014; Vasistha et al., 2019; Zecevic, Hu, & Jakovcevski, 2011). Later in pregnancy, inflammation and CRP are involved in the normal parturition process. The placenta at term synthesizes CRP (Malek et al., 2006). Increased inflammatory cytokines in the last trimester are associated with enhanced brain development, but later childhood problems with impulsivity (Graham et al., 2018; Spann et al., 2018). Maternal IL-6 levels at 16 weeks gestation, which we found associated with infant Negativity, were not similarly associated when levels across the entire pregnancy were considered (Rudolph et al., 2018). These studies did not report sex-specific effects.

The effects of choline on the development of cerebral inhibition are mediated by the activation of $\alpha 7$-nicotinic receptors (Alkondon et al., 1997; Ross et al., 2013). $\alpha 7$-nicotinic receptors on hippocampal neurons are required for the induction of the chloride ion gradient necessary for neuronal inhibition (Liu, Neff, & Berg, 2006). From Fig. 3, the effects of choline on inhibition appear to be competitive with the effects of maternal inflammation in males. In an animal model, deletion of *CHRNA7*, the gene associated with $\alpha 7$-nicotinic receptors, completely blocks the effects of choline on the development of inhibition (Stevens, Choo, Stitzel, Marks, & Adams, 2014). Direct comparison of *CHRNA7* deletion with prenatal MIA found similar effects of both insults on the offsprings' development (Giovanoli, Werge, Mortensen, Didriksen, & Meyer, 2019), providing further evidence for the possible competing effects of $\alpha 7$-nicotinic receptor activation and maternal inflammatory effects on brain development. The $\alpha 7$-nicotinic receptor activation mediates the anti-inflammatory action of the vagus nerve (Wang et al., 2003). In pregnant wild-type C57BL/6N mice subjected to MIA, choline supplementation did not change IL-6 levels in the placenta, but did lower IL-6 levels in the fetal brain, suggesting no general anti-inflammatory effect but rather a specific effect in the fetus itself. Effects were seen in both males and females (Wu et al., 2015). Effects of choline on maternal cytokine levels were observed in a rat gestational model with nearly fivefold dietary choline supplementation (Zhang et al., 2018), but there were no effects on mothers' cytokine levels observed in our study with normal human diets. A study of twofold choline supplementation on the expression of placental angiogenic factors and pro-inflammatory factors in a mouse model with a hemizygous *Dlx3*± gene deletion model of placental insufficiency found that the effects of supplementation in early gestation were greater in male placentas and in later gestation, greater in females (King et al., 2019). Thus, like MIA, the sites of choline's effects appear to be complex, with different but interacting effects in mother, placenta, and fetal brain.

Effects of choline in the human infants in this study were most marked on the development of cerebral inhibition and behavioral Regulation where sex-dependent effects of inflammation were also seen. Sex-specific effects of choline have not been generally observed in other human studies of choline's effects (Jacobson et al., 2018; Ross et al., 2013). Nonetheless, for the more vulnerable male fetus in particular, higher choline levels would seem to be a useful intervention to prevent the initial steps in the pathophysiology of later mental illness from occurring before birth in the mother who experiences inflammation. Three randomized clinical trials of choline-containing supplements have reported positive results on child cognition and behavior (Caudill, Strupp, Muscalu, Nevins, & Canfield, 2018; Jacobson et al., 2018; Ross et al., 2013, 2016). The US Food and Drug Administration recommends at least 550 mg choline daily from diet, and the American Medical Association recommends choline supplements as part of prenatal vitamins for all women (American Medical Association, 2017; Food & Drug Administration, 2016).

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