**Within & Between Functional Connectivity in Neonates is Associated with Mean Maternal IL-6**

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**INTRODUCTION**

Epidemiological evidence and work in animal models supports the link between maternal inflammation during pregnancy and an increased likelihood of mental and psychiatric disorders in offspring, including Autism & Schizophrenia.

**Cytokines**, inflammatory signaling proteins, are expressed in the fetal brain and involved in multiple stages of neurodevelopment, including cellular survival, proliferation and differentiation, neural growth and synaptogenesis.

**Intervention** is often complex and multifaceted, appearing to be particularly important in the processes leading from maternal inflammation to alterations in fetal brain development and increased risk for psychopathology.

**Relationships** between fetal brain development and maternal inflammation, largely based on animal models thus far, and no studies to date have looked at this relationship regarding human neonatal functional brain development.

**Examining brain structure and functionally shortening after birth** increases the capacity to distinguish between the influence of maternal inflammation during pregnancy from postnatal environmental factors.

**Aims: Determine Association between Neonatal Functional Brain Networks & Maternal IL-6**

1. **Assess widespread effects of IL-6 on brain development:** To gain further insight regarding wide-spread effects of elevated maternal inflammatory rates on neonatal brain development, resting state networks consistently detected in older cohorts are used to assess functional neonatal brain development in the context of elevated maternal IL-6 concentrations over time.
2. **Assess & differentiate connections within & between networks:** In the current study, in order to parse out network specific associations with IL-6, we analyze network structure separately within and between functional systems or sub-networks.
3. **Assess robustness of model-based predictions:** Machine-learning methods partnered with robust assessments of accuracy and generalizability using cross-validation and permutation testing may provide new insights into complex system-wide relationships common univariate ROI-based approaches may fail to yield.

**Methods: We Assessed 84 Maternal-Neonate Dyads Using Neonatal Functional Connectivity & Mean Maternal IL-6**

Participants: 84 neonate/mother dyads (Neonates <30 days, 50 Male/Female) 

Pre-processing steps: 
1. Maternal (during pregnancy): maternal use of psychotropic medication, maternal use of anti-convulsant, axial or congenital, genetic, or neurologic disorder of the fetus (e.g., Diaper stains, FPGs).
2. Infant: birth before 26 weeks gestation; evidence of a congenital, genetic or neurologic disorder.

IL-6 Collection:
1. Collection of maternal blood samples for measurement of IL-6 occurred in the 1st, 2nd, and 3rd trimesters.
2. Peripheral blood was drawn intrauterine and allowed to clot for 30 min (room temperature).
3. Samples were obtained using a commercial high sensitive ESKA (includes cytokine).

Scan Procedure & Image Acquisition:
1. Infants were scanned during natural sleep at UC Irvine. Waking and registration were monitored throughout the scan.
   - Single-scan state scan (6.5 minutes) on a 3T Tim Trio Siemens scanner
   - 2D Weighted EP (TR = 2.000; TE = 33ms)
   - 2D Anatomical (TR = 3300ms, TE = 255)

**Pre-processing:** Data processed & analyzed at OHSU

Neurophysiologic events (Fox et al., 2005) including removal of central spike, slice time & motion correction, intensity normalisation, bandpass filtering and registration to atlas space.

**Registration:** Images are co-registered to infant standard template at Talairach space.

**Masking:** In-house technique labeled mouse-mask was created, validated and added to our pre-processing pipeline. This process crops out extraneous non-brain tissue, then allows for fMRI Brain Extraction Tool. Results are then refined using the mask to correct registered functional data to ensure accurate results.

**Motion:** To ensure maximum reliability of fMRI signal, only subjects with at least 6 minutes of data after correction using a frame-wise displacement cutoff of 0.2 mm were included. One frame prior to, and two frames after each frame exceeding this threshold are removed from the timeseries for analysis (Power et al., 2012)

Network Analysis:
1. **6 levels** were determined using a commercial high sensitive ESKA (includes cytokine).

**Results:**

1. **Multivariate:** Partial least squares regression used to make predictions

   - Modelling complex relationships between a large subset of predictors (FG & target outcomes) 
   - Similar to Principle Components Analysis (PCA), PLSR models a response by reducing a large set of correlated features into orthogonal (uncorrelated) components accounting for the greatest amount of variance in a dataset.

2. **Predictors & Outcomes:** Unlike PCA, PLSR uses both the predictors (x) and outcomes (y) to model a response. Minimizing the relationship between predictors and maximizing covariance (prediction) between x and y (Abdi and Williams, 2013)

3. **Cross-Validation:** Internal k-fold cross-validation is used to estimate an optimal number of components on all available data. Next an independent model is fit using randomly-partitioned train/test sets using a hold-out (KHO) procedure over 4000 repetitions (Rudolph et al., under review)

4. **Model Assessment:** Each cross-validated model is tested for robustness against a random permutation (chance) over all iterations

**Conclusions & Discussion**

**Functional Topology:** FC within & amongst functional systems is associated with IL-6

- Diminishing the need to consider wide-spread effects of maternal inflammation on offspring neurodevelopment

**Implications for Development:** Associations of mean maternal IL-6 with between network connectivity involves networks and regions previously identified as important for supporting normative social, emotional and cognitive development.

**Relevance for Neuropsychiatric Disorders:** Sensorimotor (SSM) & Salience (SAL, SUB) systems display significant associations with frontal pole (FP) and dorsal attention (DAN) cognitive control networks (SAL, SUB, FP) associated with IL-6 during pregnancy.

**Figures:**

- **Within Network:** VIS, SAL, DAN
- **Between Network:** SAL, VIS, CON, SSM, FP, SUB

**Spotlight on functional connectivity within & between the salience network with sensorimotor & cognitive control systems**

**Funding:** NIMH