WITHIN & BETWEEN FUNCTIONAL CONNECTIVITY IN NEONATES IS ASSOCIATED WITH MEAN MATERNAL IL-6

A RESTING STATE FUNCTIONAL CONNECTIVITY STUDY

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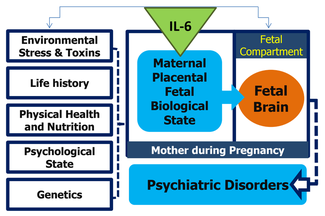
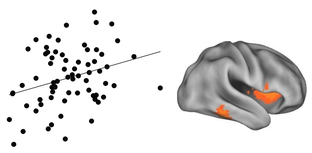
INTRODUCTION

1. **Epidemiological evidence and work in animal models** supports the link between maternal inflammation during pregnancy and an increased likelihood of multiple neurological and psychiatric disorders in offspring including Autism & Schizophrenia
2. **Cytokines, inflammatory signaling proteins**, are expressed in the fetal brain and involved in multiple stages of neurodevelopment, including cellular survival, proliferation and differentiation, axonal growth and synaptogenesis
3. **Interleukin-6 (IL-6), a pro-inflammatory cytokine** appears to be particularly important in the processes leading from maternal inflammation to alterations in fetal brain development and increased risk for psychopathology
4. **Relationship between fetal brain development and maternal inflammation** largely focused on animal models thus far, and no studies to date have looked at this relationship regarding human neonatal functional brain development
5. **Examining brain structure and functioning shortly after birth** increases the capacity to distinguish between the influence of maternal inflammation during pregnancy from postnatal environmental factors



Proposed pathways linking pre and post natal development, modified from Buss et al. 2012



Amygdala to Insula connectivity is highly associated with Mean Maternal IL-6 (Graham et al., 2014)

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)
Exclusionary criteria

i. **Mothers (during pregnancy)**: maternal use of psychotropic medication; maternal use of corticosteroids; and known congenital, genetic, or neurologic disorder of the fetus (e.g., Down syndrome, fragile X)
ii. **Infants:** birth before 34 weeks gestation; evidence of a congenital, genetic or neurologic disorder

IL-6 Collection

i. **Collection of maternal blood samples for measurement of IL-6 occurred in the 1st, 2nd and 3rd trimesters**
   i. Peripheral blood was collected in serum tubes & allowed to clot for 30 min (room temperature)
   ii. Serum IL-6 levels were determined using a commercial high sensitive ELISA (eBioscience)

Scan Procedure & Image Acquisition

Infants were scanned during natural sleep at UC Irvine. Waking and respiration were monitored throughout the scan.

i. Single resting state scan (6.5 minutes) on a 3T Tim Trio Siemens scanner
ii. T2* Weighted EPI (TR = 2.0 (2000ms); TE = 30ms)
iii. T2 Anatomical (TR = 3200ms; TE = 255)

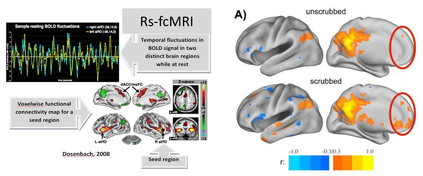


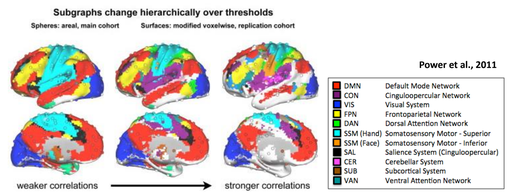
Image Preprocessing (Data processed & analyzed at OHSU)

Pre-processing steps to reduce spurious variance unlikely to be involved in neurophysiologic events (Fox et al., 2005) including removal of central spike, slice timing & motion correction, intensity normalization, bandpass filtering and registration to atlas space

i. **Registration:** Images are co-registered to **infant standard templates*** from the MRI Study of Normal Brain Development (Fonov et al., 2011; Fonov et al., 2009), then registered to Talairach atlas-space.
ii. **Masking:** In-house technique labeled *refine-mask* was created, validated and added to our pre-processing pipeline. This process crops out extraneous non-brain voxels, then utilizes FSL's® (BET)© Brain Extraction Tool. Results are then *refined* using the mask from co-registered functional data to ensure accurate results
iii. **Motion:** To ensure maximum reliability of rs-fcMRI signal, only subjects with at least 4 minutes of data after correction using a framewise displacement cutoff of 0.3mm FD were included. One frame prior to, and two frames after each frame exceeding this threshold are remove from the timeseries for analysis (Power et al., 2012)

Network Analysis

i. **264 nodes of interest** comprising multiple communities identified in a previous study (Power et. al. 2011) are used to assess associations of neonatal brain development and IL-6 using **partial-least squares regression (PLSR)**

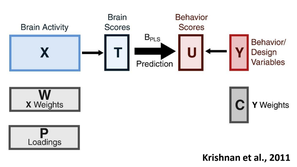


Power et al., 2011

(PLSR) PARTIAL-LEAST SQUARES REGRESSION IS USED TO MAKE PREDICTIONS

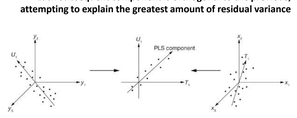
Modelling complex relationships between a large subset of predictors (FC) & target outcomes (IL-6)

i. **Multivariate:** PLSR is well suited to assess complex multivariate relationships within high-dimensional datasets (number of connections/features; Krishan et al. 2011)
ii. **Components:** 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
iii. **Predictors & Outcome(s):** 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)
iv. **Cross-Validation (CV):** 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 (%20) procedure over 4000 repetitions (Rudolph et al., under review)
v. **Model Assessment:** Each cross-validated model is tested for robustness against a random permutation (chance) over all iterations



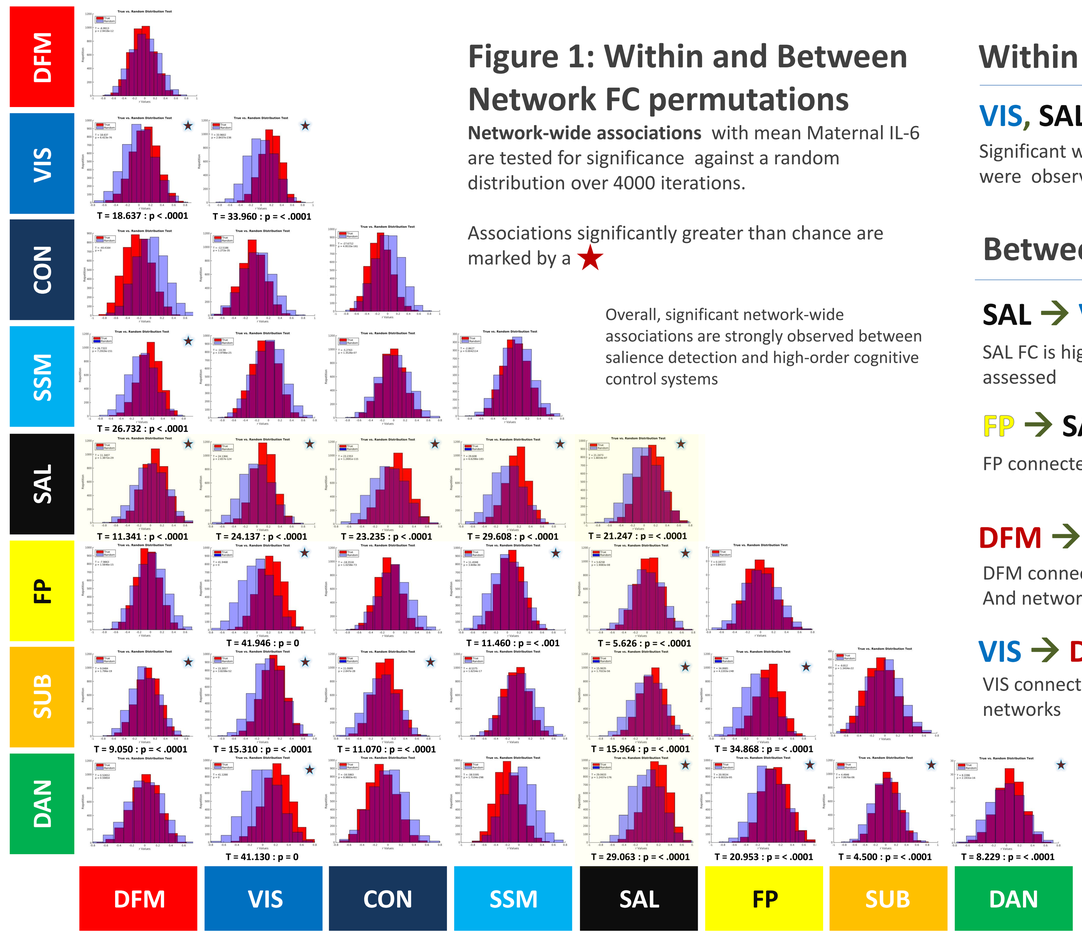
Krishnan et al., 2011

First component explains the greatest variance. Each subsequent component is orthogonal to the previous, attempting to explain the greatest amount of residual variance



Bjerrum JT *et al.* (2008)

WITHIN & BETWEEN NETWORK FUNCTIONAL CONNECTIVITY (FC) IN NEONATES IS ASSOCIATED WITH MEAN MATERNAL IL-6



* DIAGONAL = WITHIN NETWORK : OFF-DIAGONAL = BETWEEN NETWORK

Figure 1: Within and Between Network FC permutations

Network-wide associations with mean Maternal IL-6 are tested for significance against a random distribution over 4000 iterations.

Associations significantly greater than chance are marked by a ★

Overall, significant network-wide associations are strongly observed between salience detection and high-order cognitive control systems

Within Network

VIS, SAL , DAN

Significant within network associations to IL-6 were observed in 3 networks

Between Network

SAL → VIS, CON, SSM, FP, SUB, DAN

SAL FC is highly significant with most major networks assessed

FP → SAL , VIS, SSM, SUB, DAN

FP connected to salience and somatosensory networks

DFM → SAL , VIS, SSM, SUB

DFM connectivity to primary sensorimotor networks And networks involved in processing salient information

VIS → DFM, SSM, SAL, FP, SUB, DAN

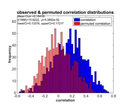
VIS connectivity to salience and cognitive control networks

Methods Comparison

Future direction**

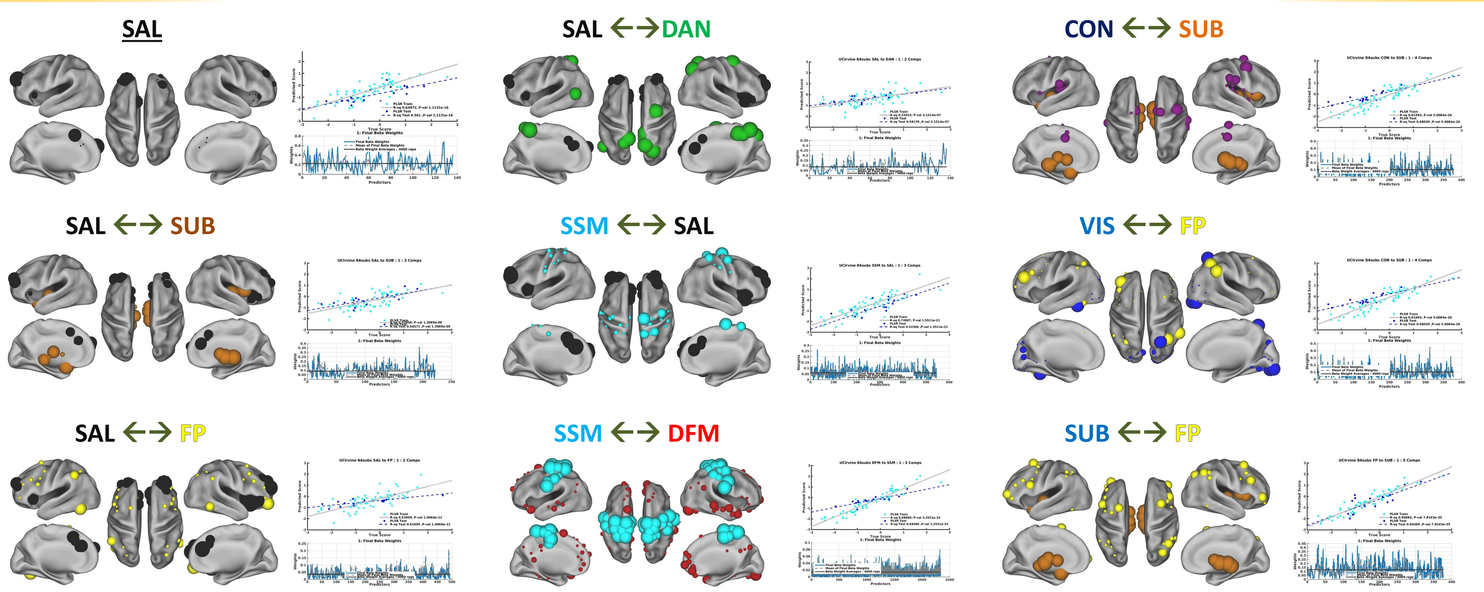
Within network FC significantly associated with IL-6 and the VIS network using a **Random Forest approach based on decision trees**

Within Network Example : VIS



NETWORKS & FEATURES ASSOCIATED WITH MEAN MATERNAL IL-6

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



* Plots represent an optimal fit for a given model after cross-validation (over 4000 repetitions) *
* Nodes are scaled according to the sum of their absolute beta-weights *

CONCLUSIONS & DISCUSSION

Functional Topology: FC within & amongst functional systems is associated with IL-6 demonstrating 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 **frontoparietal (FP) and dorsal attention (DAN) cognitive control networks** implicit in multiple neuropsychiatric disorders, including ADHD, ASD, & Schizophrenia consistent with previous work linking these disorders to maternal inflammation during pregnancy.

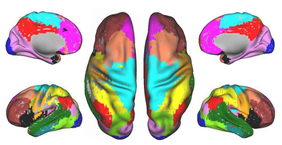
FUTURE DIRECTIONS

Interpretation: Results presented here should not be assumed to confer risk for poor outcomes. Interactions with a host of context-dependent factors in the post-natal environment may act as "second-hit" post-exposure to prenatal stressors and warrants further research

Translation & Intervention: Ultimately it will be important to assess intervention strategies aimed at reducing the impact that stressors effecting the Maternal-Placental-Fetal complex have on offspring brain development

Longitudinal Framework: **Moving forward** it will be important to track the developmental trajectories of brain topology identified here and assess their **impact on behavioral and cognitive development**

Longitudinal Framework: Emergent, but fragmented adult-like functional topology is evident by 1 YR
(Rudolph et al., 2014 – Flux Poster)



Above: Voxelwise community detection via the Infomap algorithm in a sample of 25, 1 year old infants

References: Graham et al. 2014,2015; Buss et al., 2010,2012; Estes et al. 2016; Coussons-Read et al., 2007; Knuesel et al., 2014; Doria et al., 2010; Fransson et al., 2011; Gao et al., 2011; Fair et al., 2007; Power et al., 2010, 2011, 2012; Krishnan et al. 2011; Abdi et al., 2013; Bjerrum et al., 2008; Fonov et al., 2011; Fonov et al., 2009

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