Maternal IL-6 during pregnancy can be estimated from newborn brain connectivity and predicts future working memory in offspring

Epidemiological evidence and work in animal models supports a strong correspondence between maternal inflammation during pregnancy and an increased likelihood of multiple psychiatric disorders in affected offspring, including autism, schizophrenia, attention-deficit hyperactivity disorder (ADHD) and major depression. Until relatively recently, inflammation was thought to arise purely from infection or injury; however, it is now well documented that environmental, psychosocial and general physical health factors (for example, obesity, famine, diet, low socioeconomic status, poverty and physical and/or mental stress) can elicit alterations in the immune system leading to heightened inflammation. The developing fetus receives cues about the extraterine environment via stress-sensitive aspects of maternal-placental-fetal biology, including inflammatory processes, known to act in the intergenerational transmission of environmental risk factors. Maternal inflammation during gestation has been linked to adverse outcomes during childhood and an elevated risk for psychopathology. Maternal inflammatory processes during pregnancy are therefore of significant interest as a potential common mediator of a wide range of prenatal stress-sensitive aspects of maternal-placental-fetal biology, including inflammatory processes, known to act in the intergenerational transmission of environmental risk factors. Maternal inflammation during pregnancy is unlikely that downstream of effects of maternal inflammation to the developing fetus, which can then lead to altered social and cognitive behaviors in affected offspring.

We treated IL-6 as an indicator of overall maternal systemic inflammation with potential to influence placental and fetal inflammatory processes and subsequently fetal brain development in concert with other important inflammatory mediators. Research suggests that higher systemic levels of inflammatory markers, including IL-6, may lead to cognitive and behavioral deficits in affected offspring by altering the formation of synapses and affecting synaptic function. Disruption in normal synaptic signaling and transmission has been shown to alter the balance of neurotransmitters and the number of excitatory versus inhibitory connections in the developing brain, potentially setting the stage for a diverse range of adverse developmental outcomes. Given that neuroinflammation appears to play a common role across multiple neuropsychiatric and neurological disorders and that inflammatory markers such as IL-6 are expressed throughout the brain, it appears cytokines have the potential to affect growth processes at every stage of fetal brain development. As such, it is unlikely that downstream of effects of maternal inflammation are restricted to a single brain region or canonical circuit, but are more likely to be broad.

Several lines of evidence support the link between maternal inflammation during pregnancy and increased likelihood of neurodevelopmental and psychiatric disorders in offspring. This longitudinal study seeks to advance understanding regarding implications of systemic maternal inflammation during pregnancy, indexed by plasma interleukin-6 (IL-6) concentrations, for large-scale brain system development and emerging executive function skills in offspring. We assessed maternal IL-6 during pregnancy, functional magnetic resonance imaging acquired in neonates, and working memory (an important component of executive function) at 2 years of age. Functional connectivity within and between multiple neonatal brain networks can be modeled to estimate maternal IL-6 concentrations during pregnancy. Brain regions heavily weighted in these models overlap substantially with those supporting working memory in a large meta-analysis. Maternal IL-6 also directly accounts for a portion of the variance of working memory at 2 years of age. Findings highlight the association of maternal inflammation during pregnancy with the developing functional architecture of the brain and emerging executive function.
The relationship between maternal inflammation and fetal brain development has largely focused on animal models as a result of various methodological limitations. While this work is of critical importance, particularly for testing causal models and mechanisms of action, more studies evaluating associations between maternal inflammation and neurodevelopment in human offspring are needed to understand the relevance of this work for human health. Noninvasive neuroimaging methodologies are critical toward this end. Importantly, noninvasive functional neuroimaging allows investigators to examine large-scale distributed systems across the brain as they form and are modified during development.

Considering the ubiquitous role of IL-6 and other inflammatory processes in the CNS, such an approach is needed to identify and characterize effects on brain development. As a complex system, the brain exhibits systems properties that are conserved across biological and nonbiological systems alike. One such feature is the degree to which the brain is organized into modular subsystems (i.e., communities or networks) that integrate to support complex behavior and cognition. Several studies have now shown that when communication within or between these systems is disrupted, deficits in cognitive performance, atypical behaviors and pervasive neurodevelopmental disorders can ensue.

Executive function is a broad term that describes a set of cognitive processes that support goal-directed behavior. In adults and children, executive function has been shown to rely on large-scale, distributed brain systems, such as those examined in the present study. Working memory, specifically, is a resource-limited executive function that relates to the ability to temporarily hold items in mind for manipulation. It is a core component of executive function that can be reliably measured beginning at 2 years of age. At these early ages, working memory performance serves as a foundation for later emerging academic skills, social skills and theory of mind. It is also relevant for long-term clinical outcomes, with deficits apparent across psychiatric disorders linked to inflammation during pregnancy, including ADHD, autism and schizophrenia. We therefore hypothesized that heightened maternal inflammation during pregnancy would have implications not only for large-scale functional brain systems in the neonatal period, but also for subsequent emerging working memory skills. We examined working memory as a starting point for understanding the implications of maternal inflammation during pregnancy and associated alterations in early brain system development for subsequent core cognitive competencies. However, in line with our understanding that inflammation has potential to broadly affect developing neural systems, we conceptualize working memory as only one of several aspects of executive function that may be altered in association with heightened maternal inflammation during pregnancy.

We utilized resting-state functional connectivity magnetic resonance imaging (MRI), network-based analytics and multivariate machine-learning methodologies to investigate associations between inflammation during pregnancy (indexed via maternal IL-6 concentrations in early, middle and late gestation) and newborn functional brain network topology. We posit that, if maternal inflammation during pregnancy is highly relevant for fetal development of large-scale multivariate brain systems, then it should be possible to infer (i.e., estimate) levels of maternal inflammation on the basis of large-scale brain connectivity patterns soon after birth. Furthermore, if maternal IL-6 concentrations during pregnancy are relevant for future working memory performance, and IL-6-related alterations in neonatal functional connectivity underlie this association, then (i) the brain regions that most strongly contribute to the model's ability to estimate IL-6 (i.e., are more heavily weighted) are likely to overlap with brain systems known to be involved with working memory and (ii) maternal IL-6 levels in our sample should relate to future working memory itself. Thus, we assessed the multivariate relationship between maternal IL-6 levels concentrations during pregnancy and newborn functional brain connectivity within and between previously identified large-scale brain networks. Further, we examined the correspondence of features identified within these multivariate brain models to a meta-analysis of the working memory literature in a large number of studies using Neurosynth. Last, we tested the association between serial measurements of IL-6 throughout pregnancy and working memory at 2 years of age. The results provide strong evidence linking maternal inflammation during pregnancy with newborn brain organization and future executive function.

Results
To assess the relationship between mean maternal IL-6 and newborn functional brain connectivity within and between systems, we harnessed a machine-learning approach along with random resampling to estimate generalized model performance (Fig. 1). Machine learning involves generating a multivariate model that reflects the underlying patterns of out-of-sample data. In the cognitive neuroscience literature it is often used with cross-validation or random resampling (as here and elsewhere) and is well suited for modeling the high-dimensional nature of brain-connectivity data for the purposes of estimating (or predicting) a univariate outcome (for example, IL-6), even within an individual subject. Thus, our first aim was to determine whether enough information exists in newborn functional connectivity data at the systems level to estimate levels of maternal IL-6 during the prenatal period.

We tested several potential confounding variables before the analysis. Despite the tight window in which data were collected (see Methods), in order to ensure effects reported in the study were not due to differences in length of gestation or maturation, we show here that mean maternal IL-6 is not associated with gestational age at birth ($r = 0.073$, $P = 0.631$), age at MRI scan ($r = 0.193$, $P = 0.200$), nor age at working memory assessment (24 months; $r = 0.087$, $P = 0.563$). Additionally, as maternal age may influence inflammatory processes and offspring neurodevelopmental outcomes, we examined the associations between maternal age and IL-6 levels ($r = 0.037$, $P = 0.870$) and between maternal age and infant working memory at 2 years of age ($r = 0.040$, $P = 0.800$). The results suggest that these various factors are unlikely to serve as confounds in these analyses.

To examine associations between neonatal functional brain connectivity within and between previously identified networks and mean maternal IL-6 (see Methods), we first extract pairwise functional connections (correlations) within or between regions of interest (ROIs) of ten common and previously defined functional brain networks: the default mode (DFM), visual (VIS), cingulo-opercular (CON), sensorimotor (SSM), salience (SAL), frontoparietal (FP), subcortical (SUB), dorsal attention (DAN), ventral attention (VAN) and cerebellar (CER) systems. Thus, a full cross-correlational matrix is created for every participant ($264 \times 264 \times 84$). Next (Fig. 1), the connections for a given network (10 within, 45 between) across participants are used as input features to estimate maternal IL-6 concentrations using partial least-squares regression (PLSR). Examining connections by network increases interpretability of findings while also facilitating feature reduction. While these networks and their corresponding regions of interest are derived from work with adults, we posit that they are relevant to the organization of the newborn brain. Several studies have identified putative precursors of these networks in the neonatal period and have documented rapid development during infancy such that they resemble adult networks by 2 years of age.

As described further in the Methods section and elsewhere, PLSR models are generated from a subset of participants (training set) and model parameters ($\beta$ weights) are reapplied to functional connectivity data derived exclusively from a separate test set of participants (i.e., participants not used to construct the model) over

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Fig. 1 | Methods overview for combining resting-state functional connectivity MRI, random resampling and PLSR. a, b. A step-by-step overview visually depicting the process of associating neonatal functional connectivity data with mean maternal IL-6. After standard preprocessing steps, for each individual neonate, functional time courses representing regional activation for a given ROI are extracted and pairwise cross-correlation matrices are constructed for 264 regions as described by Power et al.33. From here, individual subnetworks are extracted; specifically, matrices are extracted for each of the ten networks assessed within (a) and between (b) previously identified large-scale systems (DFM, VIS, etc.). Connections between ROIs for a given within-network or between-networks functional connectivity matrix are used as features to estimate mean maternal IL-6 using partial-least-squares regression (PLSR). Using a repeated (k = 4,000) hold-out random resampling procedure, the data are randomly partitioned into training (80%) and test (20%) sets, and the resulting distribution of actual versus predicted IL-6 values is tested for significance against a null distribution (i.e., random chance).

Many iterations. To assess performance of these models in the context of the main question, permutation testing is used whereby the process is repeated and the outcome (or response variable; i.e., IL-6) is shuffled (or permuted) on each round of random resampling5. Finally, the resulting two distributions (true and random) of correlation values (index of model accuracy) are examined. An initial filtering of the strongest relationships for each within-network and between-networks model with maternal IL-6 is then conducted simply by highlighting those with P < 0.001 using a Kolmogorov–Smirnov (KS) test corrected for multiple comparisons (Table 1). However, because of the difficulty in interpreting P-values for random resampling or cross-validation tests in machine learning36,37, the primary outcome measure of interest for each model is the effect size (the amount of divergence between the true and random distributions), with larger effect sizes indicative of greater accuracy in estimating maternal IL-6 levels. Thus, we further examined only networks exceeding a small effect size, based on accepted criteria for small (0.2), medium (0.5) and large (0.8) effect sizes. Here, to ensure the selection process is robust to the number of components used for a given model, the median effect size across a range of PLSR components was used (see Methods). Additionally, we examined the results using a nested leave-one-out cross-validation (LOOCV) procedure, an alternative method for evaluating predictive models, to provide further support for our findings (see Methods and Supplementary Table 1).

Estimating mean maternal IL-6 concentrations during pregnancy from newborn functional brain connectivity. For this initial analysis, maternal IL-6 was averaged over trimesters given the high degree of correlation among IL-6 concentrations across pregnancy (r = 0.553–0.684, P < 0.001; also see Methods). Significant associations between neonatal functional brain connectivity and mean maternal IL-6 concentrations during pregnancy were identified for multiple large-scale functional systems (Table 1 and Fig. 1). Of the ten large-scale networks assessed, connectivity within one network (SAL) and between seven network-by-network

Table 1 | Partial least-squares regression results

| Networks | Cohen’s d for optimal component model | Median Cohen’s d across all components | r | S.d. | Mean train | Mean test |
|----------|-------------------------------------|--------------------------------------|---|-----|------------|-----------|
| SUB      | DAN                                 | 1.76                                 | 0.96 | 0.01 | 0.41       | 0.20      |
| SUB      | CER                                 | 1.00                                 | 0.77 | 0.02 | 0.25       | 0.22      |
| VIS      | DAN                                 | 0.86                                 | 0.56 | 0.04 | 0.20       | 0.21      |
| SAL      | CON                                 | 0.76                                 | 0.98 | 0.00 | 0.18       | 0.20      |
| SUB      | VAN                                 | 0.66                                 | 0.73 | 0.03 | 0.16       | 0.22      |
| DAN      | FP                                  | 0.61                                 | 0.91 | 0.01 | 0.14       | 0.21      |
| SAL      | CER                                 | 0.45                                 | 0.97 | 0.01 | 0.11       | 0.23      |
| VAN      | SUB                                 | 0.42                                 | 0.33 | 0.04 | 0.10       | 0.23      |

All models significant (P < 0.001) based on Bonferroni-corrected KS test. Significant associations with mean maternal IL-6 in our sample of neonates (N = 84) were identified via the cross-validated PLSR model for multiple large-scale functional systems. Within-network and between-networks findings were filtered on the basis of (i) the KS test (with Bonferroni correction for multiple comparisons) attaining statistical significance and (ii) median effect size (Cohen’s d) across all possible components (for example, 1–20) exceeding a small effect size (>|0.2|).
Features of neonatal functional brain connectivity most strongly associated with maternal IL-6 concentrations during pregnancy.

Features of neonatal functional brain connectivity most strongly associated with maternal IL-6 concentrations during pregnancy are summarized in Fig. 3. Here, absolute β weights for a given ROI are summed across all filtered within-network and between-networks models as described in the previous section and are reflected as the diameter of the node. ROIs are scaled proportionally. This measurement is a modified version of the graph-theoretical metric node strength[^26]; thus, nodes with large diameters have connections with strong influences on a given model’s ability to estimate maternal IL-6 concentrations during pregnancy, and nodes with small diameters do not. Regions in the SAL, DAN, and SUB appear to dominate the landscape (Fig. 3). Similar findings were observed when using LOOCV (Supplementary Fig. 1 and Supplementary Table 1). Supplementary Table 2 lists the unscaled absolute β weights summed for each ROI (i.e., node strength) for models estimating maternal IL-6.

**Brain regions associated with working memory overlap features more heavily weighted in models that estimate IL-6.** As noted above, we posited that if these newborn multivariate brain models (i.e., weighted predictions derived from neonatal brain connectivity) estimating maternal IL-6 are relevant for future working memory, we should be able to validate the models by testing their correspondence to regions known to be involved in working memory. To do this we began with a meta-analysis of 901 functional MRI (fMRI) working memory studies to identify the regions most tightly associated with working memory. This meta-analysis was conducted with Neurosynth software[^29]. A reverse-inference mask was generated that included all voxels in the brain that corresponded to our search term “working memory.” We did not include any additional thresholding to avoid any potential biases. Regions from our analysis were then split into those that fell inside the working memory mask (54 regions) and those that fell outside the mask (210 regions; Fig. 4b). A simple comparison of the node strengths outlined above (Table 1 and Supplementary Table 2) showed that regions within the working memory mask were significantly more strongly associated with maternal IL-6 concentrations during pregnancy than those nodes outside of the mask (Supplementary Fig. 1). While not arguing for specificity, this result suggests that altered connectivity...
correlation (maternal IL-6 and working memory at 2 years showed a negative third trimester, 0.5392). The univariate relationship between mean IL-6 measurements across each trimester directly predicted working memory performance at 2 years of age. Here, a PLSR model is generated using in 46 children in the current study who completed the Spin the Pots task at 2 years of age. Therefore, we examined working memory performance at 2 years of age in these same infants (d = 0.747) = 0.005), which establishes that increased systemic immune activation is associated with decreased working memory performance at 2 years. In this case, maternal IL-6 did not relate to offspring negative emotionality at 24 months (r = 0.003, P = 0.983). In the absence of this relationship, we tested whether newborn connectivity can predict negative emotionality directly. We did this following the same steps described in the primary analyses predicting IL-6. We identified a subset of systems that were predictive of negative emotionality; however, predictive patterns, while showing some overlap, were mostly distinct from maps predicting IL-6. Unlike the strong relationship in the original report, the overlap of regions predictive of negative emotionality with working memory regions from the meta-analysis were statistically nonsignificant (t(96) = 1.856, P = 0.415; Supplementary Fig. 3). Again, although this is not an argument for true specificity of the original findings, we believe these analyses provide another example that illustrates that our findings are not related to all behaviors.

Discussion

We highlight associations between maternal IL-6 concentrations during pregnancy (as an index of maternal inflammation) and offspring functional brain networks shortly after birth. We found
that, on the basis of these connectivity patterns within and between large-scale neural systems in the newborn brain, mean maternal IL-6 concentrations could be estimated using machine learning. This estimation capacity provides an empirical link (previously missing in humans) between prenatal exposure to inflammatory cytokines, such as IL-6, and patterns of newborn functional brain connectivity across the brain. In addition, we validate and show the potential implications of this relationship by highlighting the correspondence of brain regions estimating maternal IL-6 to regions in the brain tightly linked to working memory capacity throughout the lifespan. Last, to confirm the implications of maternal inflammation for working memory performance, we show directly that maternal IL-6 concentrations are significantly associated with working memory performance at 2 years of age.

Between-networks and within-network neonatal functional connectivity is associated with maternal IL-6. The associations observed between mean maternal IL-6 and newborn functional connectivity are widespread and involve networks and regions important for supporting normal social, emotional and cognitive development. Many of these systems also have relevance for various neuropsychiatric disorders. Specifically, the SUB, SAL and DAN systems were strongly associated with the estimation of maternal IL-6 concentrations during pregnancy. Aberrant connectivity between these networks is implicated in neuropsychiatric disorders including ADHD, schizophrenia and autism.

Within network. Within-network connectivity for one neonatal brain system, SAL, was able to estimate maternal IL-6 concentrations during pregnancy moderately. The SAL system has long been identified as being involved with detection of salient, or biologically meaningful, information, and as interacting with other brain systems to orient attention. In addition, SAL has repeatedly surfaced in the literature as being atypical across many psychopathologies. In a recent review, DiMartino and colleagues point out that system-wide changes observed in functional topology can be influenced by alterations observed in a single network. Due to the role of SAL in detecting and attending to relevant environmental stimuli and its role in engaging other cortical networks involved in executive function, it is highly plausible that individual differences in SAL could relate to altered functional topology throughout the brain, both concurrently and over the course of development. This possibility is bolstered by findings that the insula, considered a core part of the SAL system, is a particularly highly interconnected brain region beginning in early infancy.

Between network. Overall, connectivity between higher-order systems (for example, DAN, VAN, SAL) and lower-order networks (for example, SUB) were robustly associated with mean maternal IL-6 (Fig. 2, Table 1 and Supplementary Table 2). These results are noteworthy in light of prior research suggesting that individual differences in connectivity between subcortical regions and regions situated in the DAN in neonates are relevant for subsequent emotional and cognitive development during infancy. Development of and interactions between the SAL and early attention systems (for example, DAN, VAN) have also been proposed to be important for emerging effortful (or executive) control of attention. Emerging effortful control of attention involves the capacity to reorient from irrelevant to relevant stimuli before the establishment of executive control of motor output—a process that is believed to support executive functioning later on in childhood, such as task-switching and impulse control. Refinement of executive control involves an increase in communication between the DAN and later developing FP system. Our findings suggest that maternal inflammation during pregnancy is associated with the coordinated functioning between these systems as it emerges during the neonatal period.

Newborn functional connectivity is associated with regions known to be involved with future working memory, and maternal IL-6 levels directly predict working memory performance. As noted in the introduction, working memory is a core component of executive functioning that relates to emerging theory of mind, social skills, academic performance and future mental health outcomes (including ADHD, autism and schizophrenia) ADH). ADHD, autism and schizophrenia have previously been linked to maternal inflammation during pregnancy and share common deficits across a range of executive functions, including working memory performance, which may precede the traditional age at diagnosis. While the current findings in no way suggest inflammation is the ‘cause’ of these disorders, they do point to an association between at least one component dimension (i.e., atypical executive functioning and working memory) that spans each of these diagnostic domains.

Using a meta-analysis, we showed that brain regions most strongly associated with estimating maternal IL-6 also strongly overlap with regions in the brain known to be important for working memory performance. This finding implies that fluctuations in maternal IL-6 levels may be relevant for offspring working memory later in life due to associations with early emerging variation in functional brain systems. The follow-up analysis directly showed that maternal IL-6 concentrations in our sample are predictive of, and negatively correlated with, actual working memory performance in the same children 2 years later. These findings do not merely indicate an isolated association between maternal inflammation during pregnancy and offspring working memory performance; it is likely that maternal inflammation is also relevant for other cognitive domains. Indeed, working memory is known to covary with other executive functions including inhibition, task control and impulse control, among others. The brain systems capable of estimating maternal IL-6 levels in this sample (for example, CON, SAL, DAN and FP) also span a host of other higher order cognitive functions. Therefore, we assume that while our findings clearly suggest an association between prenatal inflammation and individual differences in working memory performance, the effects of prenatal inflammation are unlikely to be specific and direct causality cannot be inferred. Future translational work that takes advantage of both animal and human models in this regard will be of high importance to elucidating these issues further.

Another consideration of these findings is that maternal IL-6 acts in concert with other aspects of maternal-placental-fetal biology with potential to influence brain development and subsequent working memory. We anticipate that the amount of variance explained with regard to future working memory or other executive functions will be greatly increased by including in our model other aspects of maternal biology during pregnancy, including endocrine, metabolic and additional inflammatory markers, which also have potential to act as mediating pathways for the influence of diverse prenatal conditions on the developing fetal brain. Incorporating indicators of the quality of the postnatal environment, such as...
Limitations. The networks used to evaluate the relationship between mean maternal IL-6 and neonatal functional connectivity in the present study were originally obtained in adults, but have now been well documented across studies, age cohorts and imaging modalities\(^{14}\). Specifically, work by Gao and colleagues has previously reported the modular architecture of the infant brain is dominated by early-developing primary sensory systems, followed by the emergence of default-mode and dorsal attention systems\(^{15}\). Lin et al., using the same regions as in the current paper, have also shown that while the networks are not fully integrated at these early stages of development, their component parts are formed early on\(^{16,17}\). This approach allowed us to examine well-established and validated networks of interest and how they relate to complex behavior at 2 years of age. Nonetheless, future work using robust network definitions established during the neonatal period will be of great interest. It should also be noted that, by necessity, assessment of connectivity in the neonatal brain is conducted during sleep. While the multivariate models estimating maternal IL-6 are relatively strong and accurate during this state, more work attempting to clarify awake versus sleep patterns in infants is warranted\(^{18}\).

Our approach to identifying correspondence between the features found in the multivariate brain models and brain regions involved in working memory based on the Neurosynth meta-analysis does not indicate that these features are uniquely involved in working memory versus other cognitive abilities. Working memory itself is a well-studied phenomenon, and we chose it because it is a core component of executive function that can be reliably measured beginning at 2 years of age\(^{19}\). While our findings highlight sensitivity of maternal IL-6 and associated brain features at birth to future working memory, this finding should not be taken to imply specificity. Maternal inflammation and brain features associated at birth are likely to influence in some respect other cognitive domains as well.

With regard to the sample, the mother–infant dyads recruited and analyzed in the current study are not representative of high-risk populations, and future investigations within such samples are warranted. However, we feel analyzing maternal IL-6 in the general population, as opposed to more extreme ends of the scale (i.e., infection and/or neuro-trauma), is a particular strength of the study. This approach highlights the association of even modest variation in IL-6 with neonatal functional connectivity and later executive function. While maternal age, gestational age and age at scan were not correlated with our variables of interest (maternal IL-6 concentrations and working memory at 2 years of age), future work assessing the factors contributing to elevated maternal inflammation and interactions between pre- and postnatal factors are warranted.

Conclusion

Previous research, largely conducted in animal models, has shown associations between prenatal exposure to maternal inflammation and atypical offspring neurodevelopment and behavior. Here, using machine-learning and resting-state fMRI in a sample of 84 neonates, we show that variations in maternal IL-6 concentrations across the course of pregnancy are associated with individual differences in functional brain networks in the neonatal period and relate to future working memory performance. These results support and extend prior work examining prenatal IL-6 administration in animal models and studies at the molecular level, which highlight the role of inflammatory processes in typical and atypical neurodevelopment. Notably, by examining brain function shortly after birth, we increase the capacity to distinguish between the influences of prenatal (such as maternal inflammation during pregnancy) and postnatal environmental factors on functional brain development. Undoubtedly, pre- and postnatal environmental conditions have the potential to interactively affect brain developmental trajectories. Thus, future work should aim to characterize how pre- and postnatal factors (biological, psychosocial, environmental, etc.) interact to influence later brain and cognitive trajectories. Ultimately, such an understanding can help elucidate the complex interplay between biological transmission of risk for poor neurodevelopmental outcomes and may inform early intervention efforts aimed at reducing the impact of prenatal adversity on brain development and subsequent developmental outcomes.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41593-018-0128-y.

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Author contributions

M.D.R., A.M.G., O.M.-D., E.F., R.N. and D.A.F. drafted the manuscript and designed analyses. M.D.R. and S.E. managed data. M.D.R. performed data analysis under supervision of D.A.F. and A.M.G. C.B., S.E., P.D.W. and D.A.F. designed the study and provided insight regarding developmental models incorporating prenatal health factors. C.B., S.E. and J.M.R. provided assistance. This work was funded by the National Institute of Mental Health (grants R01 MH096773 and K99/R00 MH091238 to D.A.F.), Oregon Clinical and Translational Research Institute (D.A.F), NIMH K99 MH118105 (A.G.), the Gates Foundation (D.A.F., R.N., A.G., C.B.), the Destelzo Innovation Fund (D.A.F.), an OHSU Fellowship for Diversity and Inclusion in Research Program (O.M.–D.) and a National Library of Medicine Postdoctoral Fellowship (E.F.).

Competing interests

The authors declare no competing interests.

Additional information

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Methods

Participants. Neonates included in the study (N = 84; M = 25.45 ± d., s.d. = 12.09 ± 2.21; 50% female) are part of an ongoing longitudinal study for which mothers (N = 84; M = 28.48 ± d., s.d. = 5.13 ± 0.94) were recruited during the first trimester of pregnancy. Exclusion criteria for mothers were as follows: maternal use of psychotropic medication during pregnancy; maternal use of corticosteroids during pregnancy; maternal alcohol or drug use during pregnancy; and known congenital, genetic or neurologic disorder of the fetus (for example, Down syndrome, fragile X syndrome). Exclusionary criteria for infants were birth before 34 weeks gestation or evidence of a congenital, genetic or neurologic disorder. Our final study population of 84 mother–infant dyads came from a total of 152 mothers who were originally recruited for the study. Twenty-one mothers opted out of the MRI and DMRI scan after birth. Of the remaining 131 that were attempted, 24 were deemed unsuccessful, as no data were obtained, and 1 participant was not used because of maternal use of corticosteroids during pregnancy (which was discovered before application of the initial exclusionary criteria). The remaining 22 participants not used either did not have a successful resting-state functional MRI scan acquisition or had insufficient amounts of resting-state data (see below for more details).

All procedures were approved by the Institutional Review Board at the University of California, Irvine in compliance with ethical regulations and standards. All participants provided written informed consent. Participants with behavioral data did not differ from full sample with regard to demographic variables. These details have been provided previously. All neonates with usable MRI data and maternal IL-6 measurements were used in the current study. Simulation models incorporating effect sizes from studies on maternal stress biology at University of California, Irvine were used to guide a small variation in the neonatal brain and were used to determine the original sample size for study. While no formal statistical analysis was done to determine sample size for this specific analysis, our sample is similar in size to those of prior infant functional brain imaging work and represents, to our knowledge, the largest infant longitudinal sample to date that also includes maternal prenatal immune response data. As this was a single population sample, detailed information for any specific sample manipulations, no participant randomization was conducted during sample collection.

Maternal IL-6 collection and assessment. Collection of maternal blood samples for measurement of IL-6 occurred in early, middle and late pregnancy. Mean gestational age in weeks at each collection was 12.7 ± d. (s.d. = 2.13), 20.5 ± d. (s.d. = 1.39), 30.4 ± d. (s.d. = 1.33) for each time point, respectively. To determine IL-6 concentrations, peripheral blood was collected in serum tubes (BD Vacutainer). Serum samples were allowed to clot for 30 min at room temperature and were centrifuged at 4°C at 1,500g. Serum was then separated and stored at −80°C. Serum IL-6 levels were determined using a commercial ELISA (Biosciences) with a sensitivity of 0.03 pg/ml according to the manufacturer's instructions. The intra- and inter-assay coefficients of variability for IL-6 measurements were 10% and 14%, respectively. Measurements for the imaging portion of the analysis were averaged across trimesters, given that IL-6 concentrations at each time point were highly correlated (r = 0.553 ± 0.684, P < 0.001).

MRI data acquisition. Neuroimaging data were collected during a tight window at approximately 4 weeks of age (M = 3.79 ± d. weeks, s.d. = 1.84) during natural sleep on a TIM Trio, Siemens Medical System 3.0 T scanner. Neonates were swaddled and fitted with ear protection to reduce scanner noise. Waking and respiration were monitored. High resolution T2-weighted (TR = 3,200 ms, echo time = 255 ms, resolution = 1 x 1 x 1 mm, 4.181 mm) and T1-weighted scans (MP-RAGE TR = 2,400 ms, inversion time = 1,200 ms, echo time = 3.16 ms. flip angle = 8°, resolution = 1 x 1 x 1 mm, 6.18 mm) were collected. Functional images for resting state functional connectivity MRI (rs-fcMRI) were obtained using a gradient-echo, echoplanar imaging (EPI) sequence sensitive to blood oxygen level–dependent (BOLD) contrast (TR = 2,000 ms, TE = 30 ms, FOV = 220 x 220 x 160 mm, flip angle = 77°). Full brain coverage was obtained with 32 ascending-interleaved 4-mm axial slices with a 1-mm skip. Steady-state magnetization was assessed after 4 frames (8 s). Functional data were acquired in a single scan consisting of 195 volumes for all but eight participants, whose scans consisted of 150 volumes during the initial phase of the study.

MRI and DMRI data preprocessing. Brain images were separated from the rest of the head tissue with the Brain Extraction Tool from the FMRIB Software Library (FSL) and additional refinement with an in-house technique (labelled refine mask) to improve brain masks as necessary. This technique utilizes the mask generated from co-registered functional data back-registered to the anatomical image to ensure accurate functional images were preprocessed to reduce artifacts using tools from FSL and the 4dip Suite of Image Processing Programs (http://imaging.wustl.edu/pub/raichlab/4dip_tools/)46,48. These steps included (i) removal of a central spike caused by MR signal offset, (ii) correction of odd versus even slice intensity differences attributable to interleaved acquisition without gaps, (iii) realignment, and (iv) intensity normalization to a whole-brain mode with normalization of the mean intensity of all functional data, and division by the mean intensity of each participant via the high-resolution T2 scan. The transformation involved calculation of a single matrix for each individual to facilitate registration both to a standard infant template (0- to 2-month age range; MRI Study of Normal Brain Development) and to the Talairach coordinate system (by aligning the infant template to a custom atlas-transformed target template (711-2B) using a series of affine transforms. Each run was then resampled in atlas space, combining realignment and atlas transformation in one interpolation. All subsequent operations were performed on the atlas-transformed volumetric time series.

rs-fcMRI preprocessing. Additional preprocessing steps were employed to reduce noise and provide non-radiance stemming from white matter activity. (i) removal of a central spike caused by MR signal offset, (ii) correction of odd versus even slice intensity differences attributable to interleaved acquisition without gaps, (iii) realignment, and (iv) intensity normalization to a whole-brain mode with normalization of the mean intensity of all functional data, and division by the mean intensity of each participant via the high-resolution T2 scan. The transformation involved calculation of a single matrix for each individual to facilitate registration both to the atlas-transformed target template (711-2B) using a series of affine transforms. Each run was then resampled in atlas space, combining realignment and atlas transformation in one interpolation. All subsequent operations were performed on the atlas-transformed volumetric time series.

Partial least-squares regression (PLSR). We chose to use PLSR to assess associations between neonatal functional brain connectivity and variations in mean maternal IL-6 due to the high-dimensional feature space (number predictors). PLSR is a multivariate technique similar to principal component analysis (PCA) that models a response by reducing a large set of correlated features into orthogonal (uncorrelated) components. However, PLSR takes the outcome variable of interest (y; maternal IL-6) into consideration by limiting the relationship (amount of covariance) between the predictor variables (x) and maximizing covariance (prediction) between x and y via singular value decomposition (SVD).

Applying PLSR to within-network and between-networks connectivity matrices. Here, x represents an n-by-m two-dimensional input matrix where n is the number of participants (rows) and m is the number of connections (columns) within a given functional matrix (within or between network). y is a one-dimensional vector containing our outcome measure of interest (mean maternal IL-6) for each participant. We used cross-validation to identify the optimal number of components used to estimate mean maternal IL-6 in our sample of 84 neonates. Cross-validation is an iterative process whereby a sample dataset is randomly partitioned into training sets used (exclusively) to build the models and independent test sets used to assess a model's robustness, prevent overfitting and increase generalizability to unseen data. This approach identifies a given number of components capable of providing the best overall fit while simultaneously reducing the mean-squared error and explaining the greatest variance. To avoid selection bias and maximize sample size and generalizability within our dataset in the absence of a true validation set, we used tenfold cross-validation to estimate an optimal number of components to use per network model. With that said, to be sure that our findings were robust to this model selection step, we ran a subsequent analysis with the component selection that we used in this case, with the results being the same for a large number of components (for example, 1-20) and took the median effect size of those predictions. Thus, the procedure is agnostic and does not require the original component selection step. Findings from both procedures are shown in Table 1. Due to the nature of the analysis, data collection and analytics were not performed blind to the conditions of the experiments.

Random resampling. Using a fixed number of components, or across a range of components as identified in the previous step for a given network model, a holdout procedure is used to generate a distribution of correlations between true and estimated maternal IL-6. Specifically, here participants are pseudorandomly partitioned using a 20% holdout procedure resulting in 80% training (68) and 20% test (16) sets. This process is repeated over a large number of iterations (N = 4,096) to reduce sampling bias. The distribution of correlations (fit) between true and estimated mean maternal IL-6 is then tested for robustness against a null distribution (i.e., random chance). To achieve this, a process identical to that of
Predictive features. For each participant, a functional connectivity matrix is generated from a set of 264 ROIs that belong to larger networks or communities\(^1\) (Fig. 1). Of these previously identified networks, we assess ten commonly cited and well-validated functional brain networks: the default mode (DFM), visual (VIS), circulo-opercular (CON), sensorimotor (SSM), salience (SAL), frontoparietal (FP), subcortical (SUB), dorsal attention (DAN), ventral attention (VAN) and cerebellar (CER) systems. From the larger functional connectivity matrices comprising all 264 ROIs and networks, within-network and between-subnetwork matrices of interest are extracted, and the unique connections between ROI pairs are used as features \(x\) in the PLSR models used to estimate mean maternal IL-6. The \(\beta\) weights obtained, signifying the importance of a particular connection between ROIs in the model, were ranked and summed by their absolute values across tests (consensus features). ROIs were then plotted on a standardized brain surface using Caret 5 software (University of Washington, St. Louis) and scaled proportionally by their absolute \(\beta\) weights.

Infant working memory performance. Spin-the-pots\(^2\) is a visuospatial, multi-location search task designed to probe working memory in toddlers and young children. Pots are arranged on a spinning tray (‘lasy susan’) and participants are asked to place stickers inside 6 of 8 pots. Participants must try to remember which pots have stickers in them and choose one after each time the tray is spun. Scoring is calculated by taking the total number of possible trials (16) minus the number of errors (turns taken to recover the stickers unsuccessfully). Of the 84 neonates with resting-state functional connectivity data, to date 46 (\(M = 24.66\) months, s.d. = 0.73 months; 48\% female) have been assessed with this measure at 2 years of age. Using the same procedure as in the primary analyses (i.e., PLSR paired with random resampling), associations between maternal IL-6 concentrations in early, middle and late pregnancy and infant working memory performance were tested. Further, a traditional regression analysis was used to assess the direction (positive or negative) of the relationship between mean maternal IL-6 and working memory in our sample of neonates.

Infant negative emotionality. A revised parent-report measure of infant temperament, the Infant Behavior Questionnaire (IBQ), was used to assess infant negative emotionality\(^6\).

Nested leave-one-out cross-validation (LOOCV). We repeated the validation process described above using a leave-one-out approach. Here, the number of folds is equal to the number of participants (\(N = 84\)). Within each fold, a test participant (\(N = 1\)) is held out and predictive models are constructed using a nested LOOCV with the training data of remaining participants (\(N = 83\)). Again, to test against a null distribution, this process is repeated many times. Here, because of the computing load of generating random bootstrap models with nested LOOCV, we ran only 100 permutations to test against. Importantly, the networks we focus on in the manuscript (SUR, DAN, SAL) tend to remain the best performing models compared to random chance. In addition, the nodes most strongly related to IL-6 using the LOOCV procedure continue to overlap more strongly with regions activated in the working memory meta-analysis using Neurosynth (Supplementary Fig. 1).

Of note, LOOCV approaches tend to produce less reliable estimates of model performance, which is one reason we chose the random resampling procedure noted above. There are several limitations to the LOOCV with respect to generalizability. For example, early simulation studies\(^7\) evaluated a range of methods for model validation. These reports found an interesting relationship between the number of folds (twofold to LOOCV were tested) and the variability of the predictive error. As the validation procedure uses increasingly more data (i.e., becomes more LOOCV), the models themselves become more stable because the structure of the training data is increasingly similar across the models. However, this is not necessarily a positive result because there is very little ability to improve a poorly generalizable model (i.e., it comes at a cost to the testing data, where the testing error becomes highly variable across the folds). As a result, the predictive estimate of model performance—the mean accuracy across all folds, which is an estimate of the generalization error—becomes more variable as well. In other words, the generalizability of the performance of the model (i.e., whether this model will work with new training data) is more difficult to assess with a LOOCV procedure, as it exhibits a large pessimistic bias. This particular behavior is now well documented in the literature and discussed to some degree in the imaging field at [http://www.russpoldrack.org/2012/12/the-perils-of-leave-one-out.html](http://www.russpoldrack.org/2012/12/the-perils-of-leave-one-out.html). Again, this is one reason why we chose our current approach. Nonetheless, our general findings in the main manuscript replicate with this cross-validation procedure as well.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Code availability. Partial least-squares regression is available as a standalone function within the Matlab (The MathWorks, Inc., Natick, Massachusetts, United States) software package. Custom Matlab code used within the manuscript for all analyses is available from the corresponding author upon reasonable request.

Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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| Clearly defined error bars                                          | ☑         |         |
| State explicitly what error bars represent (e.g. SD, SE, CI)         | ☑         |         |

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection: (FSL) FMRIB Software Library and the Caret 5 and 4dfp tools from the Suite of Image Processing Programs (ftp://ftp.imaging.wustl.edu/pub/raichlab/4dfp tools/) were used for MRI data processing.

Data analysis: Matlab 2014b (The MathWorks, Inc., Natick, Massachusetts, United States), Gephi 0.9.2 network visualization software

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Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

Simulation models incorporating effect sizes from studies on maternal stress biology at University of California Irvine, and data regarding variation in the neonatal brain (Knickmeyer et al., 2008) were used to determine the original sample size for study.

Data exclusions

Our final study population (N=84) came from a total of 152 mothers who were originally recruited for the study: 21 mothers opted out of the scan after birth, of the 131 that were attempted, 24 were deemed unsuccessful, as no data were obtained, 1 participant was not utilized because of maternal use of corticosteroids. The remaining 22 participants not utilized either did not attempt a functional scan, was not successful with the functional scan, or had insufficient amounts of resting-state data (see below for more details).

Replication

Machine learning models such as those used within the current study are designed to maximize reproducibility. This is the case because subjects from the data analytics standpoint are split into multiple training and test sets, as opposed more traditional univariate models that simply utilize all of the data for a given outcome. In addition, we utilized several independent meta-analyses from a publicly available database to show our findings conform to the expected brain imaging outcomes. These attempts were all largely successful.

Randomization

As this was one normative sample without any specific sample manipulations, no participant randomization was conducted during sample collection.

Blinding

Due to the nature of the analysis, data collection and analytics were not performed blind to the conditions of the experiments.

Materials & experimental systems

Policy information about availability of materials

n/a Involved in the study
- Unique materials
- Antibodies
- Eukaryotic cell lines
- Research animals
- Human research participants

Human research participants

Policy information about studies involving human research participants

Population characteristics

Neonates included in the study (N=84; M=25.45 days, SD=12.09 days; 50% Female) are part of an ongoing longitudinal study at the University of California-irvine, for which mothers (N=84; M=28.48 years, SD=5.15 years) were recruited during the first trimester of pregnancy. The research sample is central to our primary question concerning the role of inflammation on developing functional brain systems and future executive functioning.

Method-specific reporting

n/a Involved in the study
- ChIP-seq
- Flow cytometry
- Magnetic resonance imaging
## Magnetic resonance imaging

### Experimental design

| Design type | Resting-state functional connectivity |
|-------------|--------------------------------------|
| Design specifications | 1 scan session (6.5 minutes) |
| Behavioral performance measures | Behavioral performance was not measured during resting-state scanning. |

### Acquisition

| Imaging type(s) | Functional |
|-----------------|------------|
| Field strength | 3.0 T |
| Sequence & imaging parameters | High resolution T2- (TR=3200 ms, echo time=255 ms, resolution=1x1x1 mm, 4.18 mins) and T1-weighted scans (MP-RAGE TR=2400 ms, inversion time=1200 ms, echo time=3.16 ms, flip angle=8°, resolution=1x1x1 mm, 6.18 mins) were collected. Functional images for resting state functional connectivity MRI (rs-fcMRI) were obtained using a gradient-echo, echoplanar imaging (EPI) sequence sensitive to blood oxygen level-dependent (BOLD) contrast (TR=2000 ms, TE=30 ms, FOV=220x220x160mm, flip angle = 77°). |
| Area of acquisition | Full brain coverage was obtained with 32 ascending-interleaved 4 mm axial slices with a 1 mm skip. |
| Diffusion MRI | Not used |

### Preprocessing

| Preprocessing software | FSL, Caret5, 4dfp. |
|------------------------|-------------------|
| Preprocessing steps | Brain images were separated from the rest of the head tissue with the Brain Extraction Tool (BET) from the FMRIB Software Library, and an additional refinement with an in-house technique (labeled refine mask) to improve brain masks as necessary. This technique utilizes the mask generated from co-registered functional data back-registered to the anatomical image to ensure accurate results. Functional images were preprocessed to reduce artifacts. These steps included: (i) removal of a central spike caused by MR signal offset, (ii) correction of odd versus even slice intensity differences attributable to interleaved acquisition without gaps, (iii) realignment, and (iv) intensity normalization to a whole brain mode value of 1000. |
| Normalization | Atlas transformation of the functional data was computed for each individual via the high-resolution T2 scan. The transformation involved calculation of a single matrix for each individual to facilitate registration both to a standard infant template (0- to 2-month age range; MRI Study of Normal Brain Development) and to the Talairach coordinate system (by aligning the infant template to a custom atlas-transformed target template [711-2B] using a series of affine transforms. Each run was then resampled in atlas space, combining realignment and atlas transformation in one interpolation. All subsequent operations were performed on the atlas-transformed volumetric time series. |
| Normalization template | Infant template (0- to 2-month age range; MRI Study of Normal Brain Development) and the Talairach coordinate system target template [711-2B]. |
| Noise and artifact removal | Additional preprocessing steps were employed to reduce spurious variance stemming from non-neuronal activity. Steps included: 1) regression of six parameters (head re-alignment estimates) obtained by rigid body head motion correction, 2) regression of the whole brain signal, 3) regression of ventricular signal averaged from ventricular regions-of-interest (ROI), 4) regression of white matter signal averaged from white matter ROI, 5) regression of first order derivative terms for whole brain, ventricular, and white matter signals (to account for variance between regressors), and 6) temporal bandpass filtering (0.009 Hz < f < 0.08 Hz). As described in the steps above, nuisance regression was applied prior to bandpass filtering to circumvent the potential for reintroducing unfiltered noise (i.e. previously filtered frequencies) back into the data. |
| Volume censoring | FSL, Frame-wise Displacement (FD). Additional steps were taken to examine movement of a given frame relative to the previous frame, known as framewise displacement (FD; Fair et al., 2012; Power, Barnes, Snyder, Schlaggar, & Petersen, 2012). We used a volume censoring approach, removing volumes associated with greater than .3 mm FD (and 1 preceding and 2 following volumes to account for temporal blurring; Power et al., 2012). Neonates had over 5 minutes of data remaining on average (M=5.33, S=0.072), and remaining mean FD was approximately (M=0.083, S=0.02). |

### Statistical modeling & inference

| Model type and settings | Partial least-squares regression (PLSR) is a multivariate technique similar to Principal Components Analysis (PCA) that models a response by reducing a large set of correlated features into orthogonal (uncorrelated) components. However, PLSR takes the outcome variable of interest (y; maternal IL-6) into consideration by limiting the relationship (amount of covariance) between the predictor variables (x; functional connectivity) and maximizing covariance (prediction) between x and y via singular-value decomposition (SVD). |
| Effect(s) tested | Resting-state fMRI, not task-based fMRI. |
| Specify type of analysis | Whole brain, ROI-based, Both |
Anatomical location(s)  
264 regions of interest (ROIs; 10 mm spheres) derived from a prior meta-analyses of task fMRI data and resting-state activity mapped onto a cortical surface (Dosenbach et al., 2010; Power et al., 2011, 2010).

| Statistic type for inference | ROI-based |
|-----------------------------|-----------|
| Correction                  | PLSR models estimating mean maternal IL-6 were Bonferroni corrected. Working memory maps generated via neuosynth.org are corrected for multiple comparisons using a false discovery rate (FDR) criterion of .01 |

### Models & analysis

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | Functional and/or effective connectivity |
| ☑   | Graph analysis |
| ☑   | Multivariate modeling or predictive analysis |

#### Functional and/or effective connectivity

PLSR was used to estimate mean maternal IL-6 (IV) from a region set of 264 ROIs (Power et al. 2011). Dimension reduction and prediction was done with PLSR as described above. A random resampling procedure was used whereby participants were pseudo-randomly partitioned using a 20% holdout procedure resulting in 80% training (68) and 20% test (16) sets. The holdout procedure is repeated over a large number of iterations (k=4000). The distribution of correlations (fit) between true and estimated mean maternal IL-6 is then tested for robustness against a null distribution (i.e. random chance). In order to achieve this, on each iteration the outcome variable (y) is randomly permuted. The degree to which a given model is statistically significant (i.e. greater than chance) is assessed by simply choosing networks with a p-value < 0.001 using a Kolmogorov-Smirnov (KS) test, and whose effect size is small (0.2), medium (0.5), or large (0.8).