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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	All MRI scans were acquired using a Siemens Prisma 3T whole-body scanner using the VE11 version of the Siemens software.
Data analysis	Neuroimaging data were processed using the 4dfp analysis suite v0.1.0 (https://4dfp.readthedocs.io/en/latest/) and the following software: FSL 6.0.4 (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL), MCRIB (https://github.com/DevelopmentalImagingMCRI/M-CRIB_atlas), Connectome Workbench 1.2.3 (https://www.humanconnectome.org/software/connectome-workbench), Freesurfer 7.2 (https://surfer.nmr.mgh.harvard.edu/). Following image processing, statistical analyses were conducted in R4.1.2 (https://www.r-project.org/) and MATLAB R2021b. Functions from the Brain Connectivity Toolbox103 were used to calculate measures of network segregation and integration. Freely available MATLAB code from https://github.com/mychan24/system_matrix_tools was used to calculate system segregation. Surfaces and regional effects were shown on cortical surfaces generated by MCRIB using the cifti and ciftiTools packages and Connectome Workbench 7.2. Spin tests were conducted using code from https://github.com/frantisekvasa/rotate_parcellation. Code for all analyses is publicly available at https://github.com/utooley/Tooley2023_prenatal_env_cortical_network_dev.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The derived neural and behavioral data that support the findings of this study have been deposited in Github under accession number 12785442 (https://zenodo.org/doi/10.5281/zenodo.12785442). The deidentified data (metric outputs from imaging data after preprocessing and labeled spreadsheets) from the eLABE sample will be deposited into the NIMH Data Archive repository upon conclusion of the longitudinal portion of the study, as per NIH rules and regulations. . Study analyses additionally made use of publicly available cortical atlases, including the Gordon 333-region parcellation (https://balsa.wustl.edu/2Vm69) and the sensorimotor-association axis (https://pennlinc.github.io/S-A_ArchetypalAxis/).

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	This study reports on sex assigned at birth, as determined via review of birth certificate at the first imaging timepoint. The main study sample consisted of 141 male neonates (54%) and 120 female neonates (46%, intersex was not assessed in this study). Sex was considered in all statistical analyses; we conducted analyses covarying for sex as described in the Methods.
Reporting on race, ethnicity, or other socially relevant groupings	We report data on socioeconomic status both at birth and several longitudinal timepoints. Mothers completed surveys in each trimester, at delivery, and during follow up visits every 4 months to assess social background, mental health, and life experiences. Prenatal SES was assessed using a latent factor of socioeconomic disadvantage from a confirmatory factor analysis that included measures of mother's income-to-needs ratio, educational attainment, area deprivation index, insurance status, and nutrition. Maternal self-reported highest level of education and health insurance status were collected in trimester 1. Mothers reported household income and persons in the home to calculate income-to-needs ratio in each trimester. Home addresses were collected at delivery to obtain Area Deprivation Index (ADI) percentiles; the area deprivation index is a geocoding measure that ranks neighborhoods by socioeconomic disadvantage compared with the national average based on census block data, including factors for the domains of income, education, employment, and housing quality. Average income-to-needs ratio at birth was 0.4-12.1 (M = 2.8), ADI at birth varied from 6-100 (M=67.5). Modal maternal educational attainment was high school degree/GED completed (40% of sample). Data on demographic and socioeconomic indicators (education, household income, insurance status, ADI) were also collected during follow-up visits. Prenatal disadvantage was highly correlated with disadvantage at later timepoints (see Methods).
Population characteristics	The study sample used for primary analyses consisted of 261 neonates (after exclusions, detailed below) followed longitudinally. At the neonatal time point, 261 participants (age range = 38-45 post-menstrual weeks, M = 41.3 months) were included in the analyses. The sample consisted of 141 male neonates (54%) and 120 female neonates (46%). Average gestational age was 38.9 weeks (range = 37-41.6 weeks), and average birthweight was 3274 g (range = 2200-4627 g).
Recruitment	Neonates were recruited as a part of the Early Life Adversity, Biological Embedding, and Risk for Developmental Precursors of Mental Health Disorders (eLABE) cohort, whose participants were recruited under the parent March of Dimes study. Pregnant mothers were recruited and enrolled between the second and third trimesters. Recruitment purposefully oversampled mother-infant pairs facing adversity (e.g. poverty and stress). Inclusion criteria for the study included speaking English, mother age 18 years or older, and singleton birth. Women with alcohol or other substance abuse were excluded. Neonates were excluded from the reported analyses if they had evidence of brain injury or were born preterm (<37 weeks gestational age, GA). Additional exclusion criteria included pregnancy complications (but not gestational diabetes or hypertension) and known fetal abnormalities including intrauterine growth restriction
Ethics oversight	This study was approved by the Human Studies Committees at Washington University in St. Louis and informed consent was obtained from a parent of all participants.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	The eLABE cohort is a longitudinal developmental sample, of which n = 385 neonates received an MRI scan at birth and thus comprise the

Sample size	neuroimaging subsample of the eLABE cohort. Of the 385 neonates in the neuroimaging subsample, 261 had all neuroimaging data utilized in the present study and were not subject to exclusions detailed above, so were considered for inclusion in analyses. The study therefore utilized all of the neuroimaging data at each timepoint available as part of this longitudinal neurodevelopmental sample; sample size was not chosen based on a pre-specified power analysis.
Data exclusions	Inclusion criteria for the study included speaking English, mother age 18 years or older, and singleton birth. Women with alcohol or other substance abuse were excluded. Subjects were excluded from the current analyses if they had evidence of brain injury or were born preterm (<37 weeks gestational age, GA). Additional exclusion criteria included pregnancy complications (but not gestational diabetes or hypertension) and known fetal abnormalities including intrauterine growth restriction. At each timepoint, all participants with usable functional magnetic resonance imaging (fMRI) and demographic data were included. At the neonatal timepoint, 385 neonates were scanned, and participants were excluded from all timepoints for the following reasons: <37 weeks GA at birth (n = 54), brain injury (n = 17), neonatal intensive care unit stay for > 7 days, required intubation or chest tube, antibiotics for > 3 days, cardiac disease or metabolic disorder (n = 36), birthweight < 2,000 g (n = 1), and IRB exclusion (n = 1). There were 306 participants who did not meet any of these exclusion criteria (note that some met multiple exclusion criteria). Of these participants, 261 participants (age range = 38-45 post-menstrual weeks, M = 41.3 months) were included in the current analyses at the neonatal timepoint, neonates were excluded for no usable T2 for registration (n = 27), no functional magnetic resonance imaging (fMRI) data collected or < 10 min of usable fMRI data after motion censoring (n = 12), or visible artifacts in FC data (n = 7). At the two-year time point, 202 participants were scanned, of which 162 were healthy full-term neonates not subject to the exclusions above. Participants were additionally excluded for no usable T1 for registration (n = 68) or no functional magnetic resonance imaging (fMRI) data collected (n = 2), resulting in 92 participants (range = 1.91-2.61 years, M = 2.11 years) included at year two. At the three-year point, 132 participants were scanned, of which 98 were healthy full-term neonates. Participants were additionally excluded for no usable T1 for registration (n = 31) or < 5 min of usable fMRI data after motion censoring (n = 1) resulting in 66 participants included in the current analyses from year 3.
Replication	We replicated our results with several different methodological choices and exclusion criteria, as described in the manuscript Results and Supplementary Information. All attempts at replication were successful.
Randomization	This study did not include separate experimental groups or conditions. Therefore, randomization was not performed.
Blinding	All study participants completed the same study protocol. Allocation into different experimental groups was not performed. Therefore, blinding was not needed.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☐	☒ MRI-based neuroimaging

Magnetic resonance imaging

Experimental design

Design type	Resting-state functional MRI
Design specifications	Natural sleep functional MRI, performed without sedating medications
Behavioral performance measures	No task was performed during the resting-state functional scan.

Acquisition

Imaging type(s)	T2-weighted MRI, T1-weighted MRI, resting-state functional MRI, spin-echo field map
Field strength	3T
Sequence & imaging parameters	Structural images were collected: a T2-weighted image at the neonatal timepoint (sagittal, 208 slices, 0.8-mm isotropic resolution, echo time, TE = 563 ms, repetition time, TR = 3200 ms) and a T1-weighted image at the two- and three-year timepoints (sagittal, 208 slices, 0.8-mm isotropic resolution, repetition time = 2400 ms, echo time = 2.22 ms). Resting-

state functional imaging data (fMRI) were collected using a blood oxygen level–dependent (BOLD) gradient-recalled echo-planar multiband sequence (72 slices, 2.0-mm isotropic resolution, echo time = 37 ms, repetition time = 800 ms, multiband factor = 8, 420 volumes). Spin-echo field maps were obtained (at least 1 anterior–posterior and 1 posterior-anterior) during each session with the same parameters.

Area of acquisition: Whole brain

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software: fMRI preprocessing included correction of intensity differences attributable to interleaved acquisition, bias field correction, intensity normalization of each run to a whole-brain mode value of 1,000, linear realignment within and across runs to compensate for rigid body motion, and linear registration of BOLD images to the adult Talairach isotropic atlas performed in a single step. Field distortion correction was performed, using the FSL TOPUP toolbox (http://fsl.fmrib.ox.ac.uk/fsl/ fslwiki/ TOPUP). Functions from the 4dfp analysis suite were used for preprocessing (https://4dfp.readthedocs.io/en/latest/). Following initial processing, the surface-based neonatal parcellation approach, Melbourne Children's Regional Brain Atlases (MCRIB), was used to generate surfaces for each neonatal subject and the volumetric resting-state BOLD timeseries were mapped to subject-specific surfaces using established procedures adapted from the Human Connectome Project as implemented in Connectome Workbench 1.2.3. Freesurfer 7.2 was used to generate surfaces for each toddler subject, and the volumetric resting-state BOLD timeseries were mapped to subject-specific surfaces using established procedures adapted from the Human Connectome Project as implemented in Connectome Workbench 1.2.3.

Normalization: Linear registration of BOLD images to the adult Talairach isotropic atlas space was performed in a single step. Neonates were registered: BOLD to individual T2 to group-average T2 from this cohort to 711-2N Talairach atlas. Toddlers were registered: BOLD to individual T1 to group-average T1 from this cohort to 711-2N Talairach atlas.

Normalization template: Both toddlers and neonates are registered to the 711-2N atlas, which is in standard adult Talaraich space to facilitate comparisons.

Noise and artifact removal: In the initial iteration, the data were processed with the following steps: (i) demean and detrend within run, (ii) nuisance regression including white matter, ventricles, extra-axial cerebrospinal fluid, and whole brain, as well as 24-parameter Friston expansion regressors derived from head motion. Next, frames contaminated by motion were censored as described below. Finally, the initial rs–fc preprocessing stream was repeated on the output of the initial preprocessing using only the frames that had passed motion criteria, with the addition of interpolating censored frames and band-pass filtering (0.005 Hz < f < 0.1 Hz).

Volume censoring: Neonatal fMRI data were censored at FD > 0.25 mm, with the additional restriction that only epochs of at least 3 consecutive frames FD < 0.25 mm were included. This FD threshold was selected after taking into account the smaller radius of infants' heads85 and reviewing motion traces in several subjects; respiratory filtering is unsuitable for neonatal fMRI data due to the higher respiratory rate of neonates. Toddler (two-year and three-year) fMRI data were censored based on a threshold of FDfilt > 0.2 mm, using a filtered framewise displacement trace corrected for the effect of respiration (FIRMM filtered FD), with the additional restriction that only epochs of at least 3 consecutive frames FD < 0.2 mm were included.
In order to be included in the study, a minimum of 5 minutes (375 frames) of data retained after censoring was required, though 99% of scans across timepoints had > 10 min of data retained after censoring (M = 17.4 min (1308 frames), range = 7.1-41.9 min). To account for any potential patterns of FC related to head motion or amount of data included in analyses, we calculated (i) the number of frames retained after censoring and (ii) the average FD across uncensored frames for each individual subject and included these values as subject-level covariates in in all analyses.

Statistical modeling & inference

Model type and settings: Developmental effects: A generalized additive mixed model (GAMM) was fit to each measure of cortical network segregation. GAMMs were fit with functional network segregation as the dependent variable, age as a smooth term, a random effect of participant, and biological sex assigned at birth, in-scanner motion (average framewise displacement), number of frames of fMRI retained after censoring, and average functional network weight (average network connectivity) as linear covariates. Four basis functions were specified as the maximum flexibility afforded to age splines in all models (k = 4). Models were fit using thin plate regression splines as the smooth term basis set and the restricted maximal likelihood approach for smoothing parameter selection. Random effects included a random intercept per participant.

Associations between environment and development: A GAMM was fit to each measure of cortical network segregation or to local segregation in each parcellated cortical region. We allowed the smoothed age effect in the GAMM to interact with SES; predictors thus included an age-by-SES interaction term, a smooth term for age, and covariates including sex, in-scanner motion, number of frames of fMRI, and average network weight.

Associations between outcomes and cortical segregation: A linear model was fit to estimate associations between local segregation and Bayley language scaled scores, controlling for biological sex assigned at birth, in-scanner motion (average framewise displacement), number of frames of fMRI retained after censoring, and average functional network weight.

Effect(s) tested: Developmental effects: To establish the overall magnitude of age-associated increases in cortical network segregation, we used the F-statistic for the smooth function function of age. To test for windows of significant change across the age range, we calculated the first derivative of the smooth function of age from the GAMM model using finite differences, and then generated a simultaneous 95% confidence interval of the derivative using the gratia package in R. The first derivative of this

smooth function represents the rate of change in network segregation at a given developmental time point. Intervals of significant age-related change were identified as areas where the simultaneous confidence interval of the derivative does not include zero.

Associations between environment and development: We compared two interaction models: a simpler varying coefficient (linear-nonlinear) model that allows the smooth term for age to vary as a linear function of SES, and a more complex non-linear interaction (bivariate smooth) model that allows the smooth term for age to vary as a fully non-linear function of SES. We compared models using Bayesian information criterion (BIC), and evaluated the significance of the interaction term for the selected model. All whole-brain models in the main manuscript were best fit using the simpler varying coefficient (linear-nonlinear) model that allows the linear association between SES and network segregation to vary as a smooth function of age. Interaction p-values were confirmed using a parametric bootstrap likelihood ratio test (pbkrtest package in R) for significance estimation in the mixed model context. To establish the overall magnitude of the moderating effect of SES on age-associated increases in local segregation, we used the F-statistic for the age-by-SES interaction effect.

Associations between outcomes and cortical segregation: The magnitude of associations between outcomes and measures of cortical network segregation was assessed using standardized effect sizes (βs).

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☒ Both

Anatomical location(s): Regional analyses of local segregation were conducted using the Gordon 333-region cortical parcellation, statistical models were run per ROI.

Statistic type for inference (See Eklund et al. 2016): Analyses were conducted across cortical regions, correcting for multiple comparisons across regions.

Correction: False discovery rate (FDR) correction was applied to tests conducted across cortical regions (corrected p-values across all region-wise GAMMs using FDR correction and set statistical significance at pFDR < 0.05) , and across models tested in the same set of analyses (e.g. models of age effects on cortical functional network segregation, models of age-by-SES effects on cortical functional network segregation).

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: FC was computed using Pearson correlations between regions.

Graph analysis: Weighted, signed subject-level graphs were analyzed. Measures included system segregation, the modularity quality index, the clustering coefficient (calculated at the nodal resolution), and the participation coefficient (calculated at the nodal resolution). The clustering coefficient and participation coefficient were both averaged for whole-brain analyses.