nature portfolio

Peer Review File

Prenatal environment is associated with the pace of cortical network development over the first three years of life

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

In this paper, the authors examined an important question of how SES impact longitudinal functional network development in the first years of life. The authors found low SES was associated with accelerated network segregation, but not integration, which subsequently correlated language scores at two years of age, suggesting significant impacts of SES on early brain and behavioral development. Social disparity related to SES and race has been increasingly recognized to impact early development but little is known about the brain basis underlying such impacts so the study is timely and important. There are several notable strengths of the paper including a relatively large sample size (N=261, 92, and 66 for the neonatal, 1-year, and 2-year timepoints), longitudinal design for trajectory analysis, careful imaging data quality control and processing, and multiple sets of sensitivity analysis. I do have a couple comments on the design and methodological choices.

1. SES and race are closely intertwined factors that may collectively or separately influence early brain and behavioral development. In this paper, SES was the primary variable of interest but further including race either as another variable of interest or control variable would generate useful information, assuming there is adequate racial distribution, but the current supplementary table 1 does not have this information so hard to tell.

2. Regarding the “low SES” and “high SES” separation, their definition/range were not clear in the text. A distribution of the subjects and the cutoff line over this overall measure as well as the 5 component variables would be helpful.

3. In the GAMM model, a random effect on intercept was included but not a random effect on the age term. Given the main interest of the paper on interaction between SES and age effects, this choice needs to be justified or results compared after including such a term.

4. Related, in Fig. 2, the color coding of the “dots” between the low and high SES is hard to discern to match the shaded curves. There also seem to be some outliers from high SES on the bottom of the plots that may have a driving effect, could the authors assess this?

5. I applaud the authors’ effort to map all neonatal and toddler’s dataset to the surface for further analysis, which is non-trivial given the inherently low tissue contrast in these datasets, especially for neonates. I also noticed different surface reconstruction methods were used between the neonate and later two time points and was wondering if this could have an impact on the results given the major change of segregation measures occurring mainly across this age span (i.e., better surface reconstruction results relate to higher segregation). However, I totally understand the challenges of using any alternative methods for surface reconstruction in these populations for comparison but a replication analysis using volume-based data and atlas may help.

6. Given the description that 261 total subjects were included in the analysis and 261 subjects were included in the neonatal timepoint, there should be no subjects starting at 2 or 3 years of age in their trajectory plots in all figures but this seems not to be the case. Can the authors clarify? A more detailed description of the distribution of the longitudinal timepoints for the 261 subjects (e.g., how many with all 0-2-3, how many have 0-2, 2-3, 0-3, or cross-sectional data only) would help readers better understand the dataset.

Reviewer #2 (Remarks to the Author):

This manuscript examines the development of intrinsic cortical brain networks during the first three years of life and how features of the early environment (SES) affect brain functional development. The study finds that cortical network segregation (which has been found to be associated with cognitive abilities in adolescents and adults) increases from birth to age three, with the most significant development occurring during the first two years. Prenatal SES was found to moderate the trajectory of cortical network segregation, with children from lower SES backgrounds demonstrating faster increases in segregation. These effects were most pronounced in the somatomotor and dorsal attention systems. Moreover, this study links differences in local segregation at two years of age with language and cognitive abilities, underscoring important links between early brain network development and later cognitive outcomes.

Overall, this manuscript was exceptional. The studies were elegantly designed, the research questions and analytic approaches were appropriately (and strongly) justified, and the statistical analyses were sophisticated and clean. Moreover, the paper itself was written in a clear and understandable manner. I particularly appreciated the authors' efforts to enhance transparency and reproducibility (e.g., preregistration, making code available, and excellent detail in the description of methods.). I wish every paper I reviewed was as solid as this one!

My sole comment is that I would have liked to see information provided about the race/ethnicity of the children. I think this information could help in future work seeking to extend/generalize the findings and help identify potential culturally specific relationships.

Reviewer #3 (Remarks to the Author):

This study investigated the role of prenatal socioeconomic disadvantage on the pace of cortical network development through age 3. The findings suggest that cortical network segregation increases with age, and that those from lower-SES backgrounds showed faster increases relative to those from higher-SES backgrounds. The study has several strengths, including a longitudinal sample with pre- and post-natal measures, examination of non-linear associations, and preregistered analyses. I have the following suggestions and points of clarification:

Overall, I think that the study would be clearer and more impactful with more specific framing of the construct of socioeconomic status. Variations in terminology and operationalization are a persistent challenge in this area of work, and the paper would benefit from greater clarity in this area. A clear definition of SES in the introduction would help. It seems that most of the prior work that is reviewed focuses on income, education, or neighborhood SES. However, the SES variable in the current study also includes maternal nutrition and insurance status. Is this perhaps capturing a broader construct of social disadvantage? Is maternal nutrition best treated as an indicator of social and economic status, or is it something that is predicted by social and economic status?

That said, I do appreciate the sensitivity analyses that demonstrate that the results are consistent even when SES is operationalized as just income and education.

There seems to be a large amount of attrition over time, with 261 families at the first time point, but only 66 at the third. It is clear why some participants were omitted due to exclusion criteria, but not how many participants dropped out between time points and why. In the results, there is mention of COVID-related challenges—did the authors examine whether excluded participants were significantly different from included participants in any way?

Relatedly, were any power analyses conducted?

The figures (e.g., Fig 1) seem to depict some potential outliers. For example, there is an average increase in segregation, but some children show sharp decreases in segregation, and others have especially high segregation. Were there any significant outliers? The preregistration mentions examination of outliers, but I do not think this was described in the manuscript.

What are the implications of applying adult atlases and cortical networks to infant data? This is a potential limitation that could be mentioned.

Greater attention to the underlying mechanisms would strengthen the paper. What is it about low SES that is hypothesized to alter the tempo of cortical development? This is alluded to in the

introduction, but could be more explicitly laid out. For example, are the authors conceptualizing low SES as a “harsh and unpredictable environment” or an indication of deprivation/inadequate care?

The final sentence of the manuscript is quite vague and thus does not add much to the paper: “Our results suggest that infancy and toddlerhood may be an important period for promoting healthy brain development and emphasize the first years of life as a target for policies aimed at supporting optimal child development.” If the authors wish to comment on policy implications, I think this could be done in a more thorough and specific way that is more directly linked to the data in the current study.

Reviewer #1 (Remarks to the Author):

In this paper, the authors examined an important question of how SES impact longitudinal functional network development in the first years of life. The authors found low SES was associated with accelerated network segregation, but not integration, which subsequently correlated language scores at two years of age, suggesting significant impacts of SES on early brain and behavioral development. Social disparity related to SES and race has been increasingly recognized to impact early development but little is known about the brain basis underlying such impacts so the study is timely and important. There are several notable strengths of the paper including a relatively large sample size (N=261, 92, and 66 for the neonatal, 1-year, and 2-year timepoints), longitudinal design for trajectory analysis, careful imaging data quality control and processing, and multiple sets of sensitivity analysis. I do have a couple comments on the design and methodological choices.

We would like to thank Reviewer #1 for their thorough reading of the manuscript and helpful comments. We appreciate their attention to detail and their comprehensive suggestions, which we believe have significantly strengthened the manuscript.

1. SES and race are closely intertwined factors that may collectively or separately influence early brain and behavioral development. In this paper, SES was the primary variable of interest but further including race either as another variable of interest or control variable would generate useful information, assuming there is adequate racial distribution, but the current supplementary table 1 does not have this information so hard to tell.

We agree that characterizing the racial demographics of the sample is important, and we thank the reviewer for bringing this up. We have added children's race and ethnicity to ***Supplementary Table 1***, which we have copied below for ease of review.

Supplementary Table 1. Participant demographics at birth.

Variable	N = 261[1]
Age at scan (months)	41.3 (38.0 - 45.0)
Child race	
Black	156 / 261 (60%)
Chinese	2 / 261 (0.8%)
Multiracial	3 / 261 (1.1%)
Other	1 / 261 (0.4%)
Other Pacific Islander	1 / 261 (0.4%)
White	98 / 261 (38%)
Child ethnicity	
Not Hispanic or Latino	253 / 261 (97%)
Hispanic or Latino	6 / 261 (2.3%)
Unspecified	2 / 261 (0.8%)

Child sex	
Male	141/ 261 (54%)
Female	120/ 261 (46%)
Gestational age (weeks)	38.9(37.0 - 41.6)
Birthweight (g)	3,274.0 (2,200.0 - 4,627.0)
Area Deprivation Index	67.5(6.0 - 100.0)
Income to Needs Ratio	2.7(0.4 - 9.4)
Highest level of parent education completed	
Less than 12th grade	22 /251 (8.8%)
High school degree/GED	101/ 251 (40%)
Some college/vocational school	44 /251 (18%)
College degree (4 years)	29 /251 (12%)
Graduate degree	55 /251 (22%)
Insurance status (private)	135/ 261 (52%)
Healthy Eating Index	58.7(33.0 - 80.7)
Socioeconomic disadvantage factor score	-0.1(-2.2 - 1.5)

[1]Mean (Range); n / N (%)

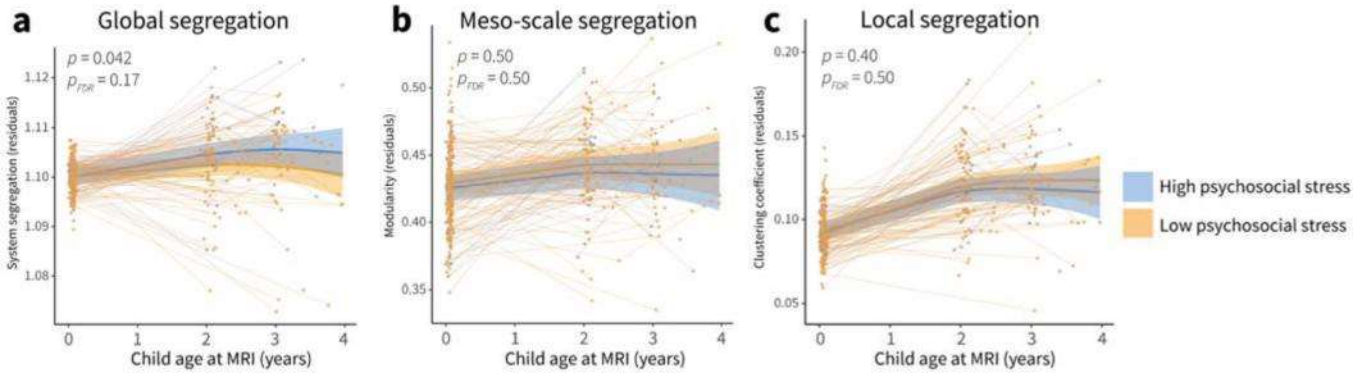
We have also added a note about how race was assessed in the ***Methods*** on page 25:

Child race and ethnicity were obtained from the child's birth certificate, options included White, Black or African American, American Indian or Alaska Native, Asian Indian, Chinese, Filipino, Japanese, Korean, Vietnamese, Other Asian, Native Hawaiian, Guamanian or Chamorro, Samoan, Other Pacific Islander, or Other for race; ethnicity was assessed by whether the child was identified as Hispanic.

Regarding including race explicitly in analyses, we note that we view race, a socially-defined construct, primarily as a proxy measure for discrimination and subsequent stress experienced by participants. We are concerned that examining participant race as a proxy, rather than directly assessing these factors, risks being interpreted in an essentialist framework and thus reinforcing the very biases that lead to discrimination and structural inequities. In this sample, we directly assessed racial discrimination using the Everyday Discrimination Scale (EDS). In prior work[1] that constructed the latent factor we used to measure social disadvantage (or SES, as we previously referred to it in the manuscript, please see response to Reviewer #3), we examined racial discrimination. We found that racial discrimination loaded primarily on a separate latent variable, that we labeled psychosocial stress, which also comprised measures of maternal depression, perceived stress, and exposure to adversity.

Of note, race was highly correlated with the social disadvantage factor indices, offering no additional improvement to the model after other variables (including racial discrimination) were accounted for; therefore, race was not included as a variable in either factor index (see Luby et al.[1]).

We agree that racial discrimination is intertwined with disadvantage (previously referred to as SES), and that the impact of structural racism as a prenatal stressor should not be discounted. However, our sample is not adequate to answer questions about the contributions of one versus the other, as in St. Louis, race and SES are highly correlated. We did examine the association of prenatal psychosocial stress with age-related changes in functional network architecture, and have added this analysis to the ***Supplement*** on page 10, copied below for ease of review:



Supplementary Figure 6. Associations between prenatal psychosocial stress and developmental increases in cortical network segregation. **a,** Prenatal psychosocial stress moderates trajectories of global cortical network segregation. **b,** Prenatal psychosocial stress does not significantly moderate trajectories of meso-scale cortical network segregation. **c,** Prenatal psychosocial stress does not significantly moderate trajectories of local cortical network segregation. Plots display fitted network segregation trajectories from GAMM models plotted by age for participants from low psychosocial stress backgrounds (orange) and high psychosocial stress backgrounds (blue). Psychosocial stress was modeled continuously; for visualization purposes here we show model trajectories from lowest and highest deciles. Individual points represent individual scans, with lines indicating scans from the same participant.

As social disadvantage and psychosocial stress are moderately correlated at birth in our sample of children ($r = 0.436$), we investigated whether there were effects of psychosocial stress independent of disadvantage. Thus, we examined whether prenatal psychosocial stress was associated with measures of functional network architecture after controlling for prenatal disadvantage. We found that when controlling for the age-by-disadvantage interaction, global segregation shows a significant age-by-psychosocial stress interaction that does not pass FDR correction ($F_{s(agexSES)} = 3.19$, $p = 0.042$, $p_{FDR} = 0.17$), while meso-scale segregation ($F_{s(agexSES)} = 0.70$, $p = 0.50$, $p_{FDR} = 0.50$) and local segregation ($F_{s(agexSES)} = 0.91$, $p = 0.40$, $p_{FDR} = 0.50$) do not show even marginally significant associations (***Supplementary Figure 6***). In all models, age-by-disadvantage interactions remain significant when including psychosocial stress in the model (p's < 0.05). We found no evidence for moderating effects of psychosocial stress on developmental changes in network integration when controlling for disadvantage ($F_{s(agexSES)} = 2.39$, $p = 0.09$, $p_{FDR} = 0.19$)

We conclude that broadly, disadvantage is more strongly associated with the development of functional network segregation than psychosocial stress, though stress may have weak and independent effects on the development of global system segregation. In turn, racial discrimination is likely not the strongest driver of the effects observed here. We have added a statement about the effects of psychosocial stress on developmental changes in cortical network segregation on page

10. It is possible that effects of psychosocial stress are only observable later in development and/or are more evident in measures of functional network integration; future work will explore this possibility.

2. Regarding the "low SES" and "high SES" separation, their definition/range were not clear in the text. A distribution of the subjects and the cutoff line over this overall measure as well as the 5 component variables would be helpful.

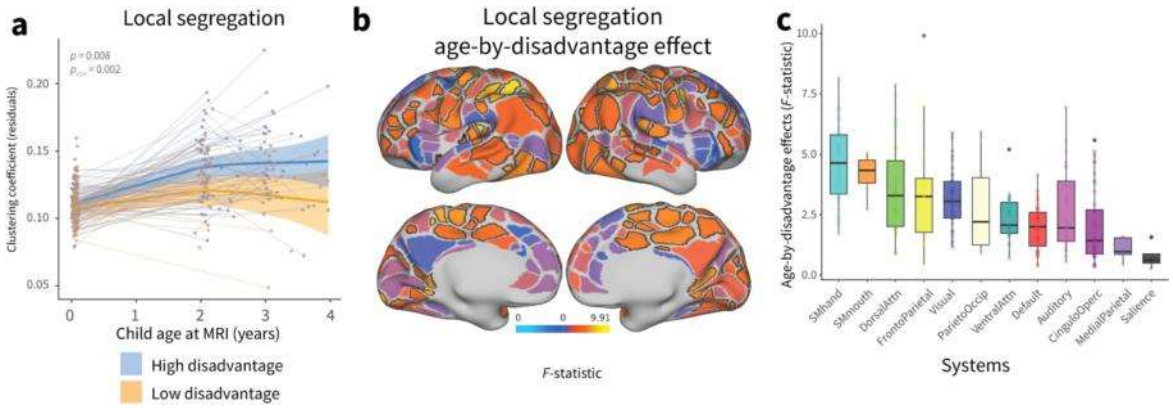
We appreciate the reviewer's focus on clarity, and apologize for the confusion. In all analyses, disadvantage (SES) was treated as a continuous variable, as dichotomizing continuous variables reduces power[2] and can lead to false positives, particularly when examining interactions[3]. For ease of visualization, in plots we present model trajectories for the highest and lowest deciles of. We have added a note to the ***Methods*** to that effect, on page 23:

While disadvantage was modeled continuously, for visualization purposes in plots we show trajectories from the highest and lowest deciles.

3. In the GAMM model, a random effect on intercept was included but not a random effect on the age term. Given the main interest of the paper on interaction between SES and age effects, this choice needs to be justified or results compared after including such a term.

We completely agree, and we thank the reviewer for bringing up this important point. We pre-registered testing only the random effect of the intercept; we were concerned about having insufficient data to fit more complex models with both a random intercept and a random effect of age, and wanted to err on the conservative side in our pre-registration. We present those analyses in the main text, but our results are qualitatively similar when also including a random effect of age.

We now include these analyses in the Supplement, in **Supplementary Figure 4** on page 8**:**



Supplementary Figure 4. Associations between the early environment and developmental increases in cortical network segregation, using a longitudinal mixed model including a random slope for child age. **a,** Prenatal disadvantage moderates trajectories of local cortical network segregation. **b,** The heterogenous patterning of the magnitude of age-by-disadvantage effects (*F*-statistic) on local segregation is shown on the cortical surface. Regions that show significant age-by-disadvantage effects passing FDR correction at $p_{FDR} < 0.05$ are outlined in black. **c,** Disadvantage effects on developmental increases in local segregation

are enriched in sensorimotor systems. Boxplots show median and interquartile range of the magnitude of age-by-disadvantage effects; each point is an individual parcel.

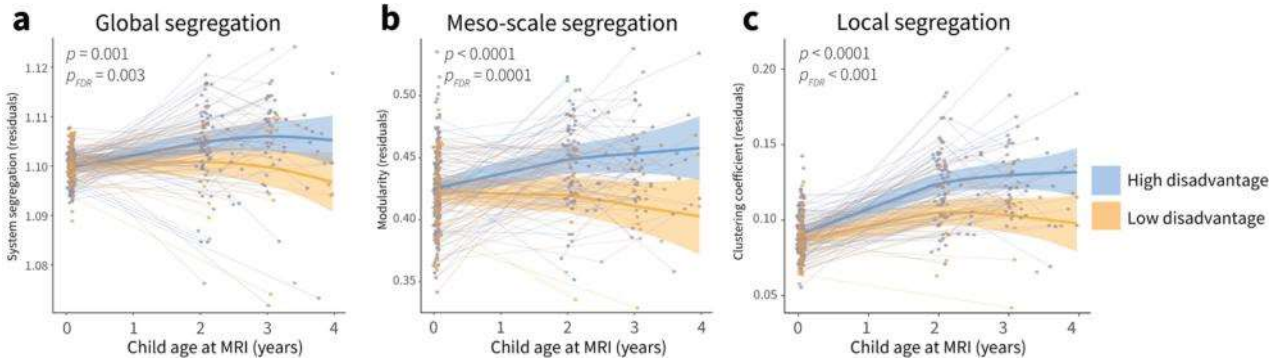
In our main analyses, we examined longitudinal development of functional network architecture using generalized additive mixed models (GAMMs), where we included a random intercept per participant. Here we include both a random slope and a random intercept per participant (uncorrelated), and find that our results are qualitatively similar. Global segregation ($F_{s(agexSES)} = 3.83$, $p = 0.02$, $p_{FDR} = 0.03$), meso-scale segregation ($F_{s(agexSES)} = 7.31$, $p = 0.0008$, $p_{FDR} = 0.002$), and local segregation (***Supplementary Figure 4a,*** $F_{s(agexSES)} = 7.17$, $p = 0.008$, $p_{FDR} = 0.002$) show similar patterns of interactions, such that infants and toddlers from more disadvantaged backgrounds show a faster increase in cortical network segregation than infants and toddlers from less disadvantaged backgrounds. There was a marginal moderating effect of disadvantage on developmental changes in network integration ($F_{s(agexSES)} = 2.49$, $p = 0.08$, $p_{FDR} = 0.08$). The magnitude of disadvantage effects on developmental increases in local segregation differed across functional systems, with the strongest effects found in somatomotor-hand, somatomotor-mouth, dorsal attention, and frontoparietal systems (***Supplementary Figure 4c***).

Additionally, we include a note in the main text referencing these analyses, on page 11:

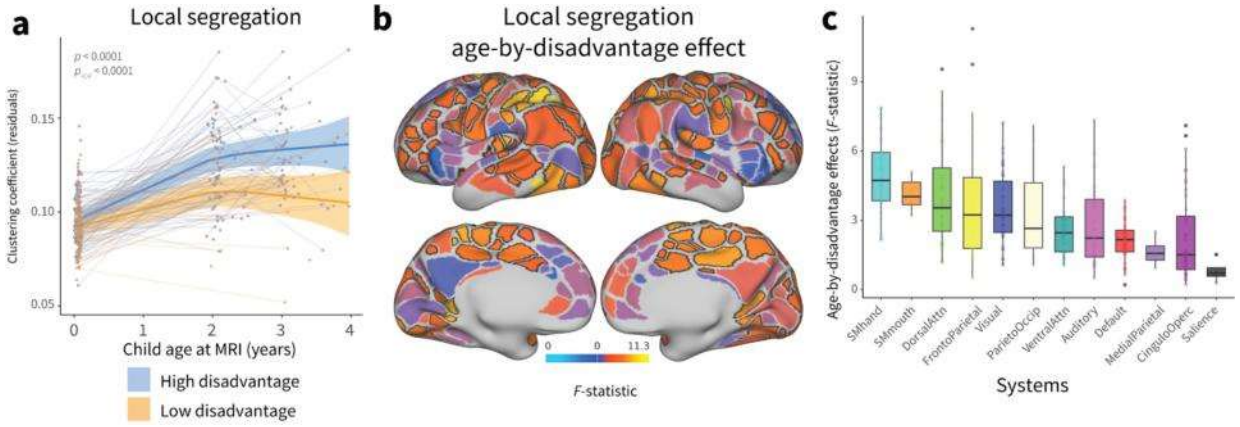
We also evaluated whether effects of prenatal disadvantage on developmental increases in cortical network segregation were accounted for by differences in sample composition over the study period, alterations in functional network architecture associated with head motion, changes in disadvantage over the study period, longitudinal modeling choices, or outliers.

4. Related, in Fig. 2, the color coding of the "dots" between the low and high SES is hard to discern to match the shaded curves. There also seem to be some outliers from high SES on the bottom of the plots that may have a driving effect, could the authors assess this?

We have modified our trajectory figures so that the confidence intervals are more transparent, and data points appear on top of the curves and best fit lines to facilitate differentiating them; the new ***Figure 2*** is copied below. We note that there is a continuous gradient of color values of the data points, as disadvantage was modeled continuously, model trajectories correspond to the fit for the highest and lowest deciles of disadvantage. Data points range from blue to blue-orange to orange, corresponding to the prenatal disadvantage of a given participant. We are happy to make additional figure modifications for clarity as needed.



Regarding outliers, in our pre-registration, we proposed several sensitivity analyses to ensure that our results were robust to methodological variation and choices about data inclusion. One of these analyses entailed excluding observations where measures of network segregation are > 3 SD away from the mean. Our results are qualitatively (and quantitatively) similar when excluding those observations. We now include this in the supplement, in ***Supplementary Figure 5***:



Supplementary Figure 5. Associations between the early environment and developmental increases in cortical network segregation, excluding outliers > 3 SD away from the mean. **a,** Prenatal disadvantage moderates trajectories of local cortical network segregation. **b,** The heterogenous patterning of the magnitude of age-by-disadvantage effects (*F*-statistic) on local segregation is shown on the cortical surface. Regions that show significant age-by-disadvantage effects passing FDR correction at $p_{FDR} < 0.05$ are outlined in black. **c,** Disadvantage effects on developmental increases in local segregation are enriched in sensorimotor systems. Boxplots show median and interquartile range of the magnitude of age-by-disadvantage effects; each point is an individual parcel.

We excluded outlier observations, that is, observations at a timepoint that were > 3 SD away from the mean, and find that our results are qualitatively similar. Global segregation ($F_{s(agexSES)} = 2.91$, $p = 0.05$, $p_{FDR} = 0.069$), meso-scale segregation ($F_{s(agexSES)} = 7.68$, $p = 0.0005$, $p_{FDR} = 0.001$), and local segregation (***Supplementary Figure 5a,*** $F_{s(agexSES)} = 13.32$, $p < 0.0001$, $p_{FDR} < 0.0001$) show similar patterns of interactions, such that infants and toddlers from more disadvantaged backgrounds show a faster increase in cortical network segregation than infants and toddlers from less disadvantaged backgrounds. We found no evidence for moderating effects of disadvantage on developmental changes in network integration ($F_{s(agexSES)} = 1.862$, $p = 0.16$, $p_{FDR} = 0.16$). The magnitude of disadvantage effects on developmental increases in local segregation differed across functional systems, with the strongest effects found in somatomotor-hand, somatomotor-mouth, dorsal attention, and frontoparietal systems (***Supplementary Figure 5c***).

5. I applaud the authors' effort to map all neonatal and toddler's dataset to the surface for further analysis, which is non-trivial given the inherently low tissue contrast in these datasets, especially for neonates. I also noticed different surface reconstruction methods were used between the neonate and later two time points and was wondering if this could have an impact on the results given the major change of segregation measures occurring mainly across this age span (i.e., better surface reconstruction results relate to higher segregation). However, I totally understand the challenges of using any alternative methods

for surface reconstruction in these populations for comparison but a replication analysis using volume-based data and atlas may help.

We thank the reviewer for their positive appraisal of our surface-mapping procedures, and for their insightful comment. To leverage leading-edge methods that maximize signal-to-noise[4] and account for individual-specific differences in brain folding, we used different methods to generate cortical surface reconstructions at the neonatal and toddler (year 2 and 3) timepoints. To generate the most accurate surfaces possible, we chose to use the highest-performing surface reconstruction method for data at each age (MCRIB for neonates and Freesurfer for toddlers), as at the moment there is no single pipeline that performs equally well across all developmental time points. This also entailed using the structural imaging modality with the highest contrast at each age (i.e., T2 in neonates and T1 in toddlers). The most salient methodological point, however, is that we use the same surface mapping procedures at all ages: the software pipeline to map the functional MRI data to the individual-specific surface is identical for all analyses. Thus, we have standardized the most salient element of the analysis pipeline highlighted by the reviewer, while still leveraging optimal age-specific tools at key earlier steps.

Regarding volume-based analyses, in other work, we have examined the impact of surface vs. volume-based analyses, finding that invariably surface-based analyses perform better. In Sylvester et al.[4], we found that surface-based analyses resulted in higher similarity between neonatal and adult brains (panel a, below), an effect that was similar to that of increasing data quantity (panel b, below).

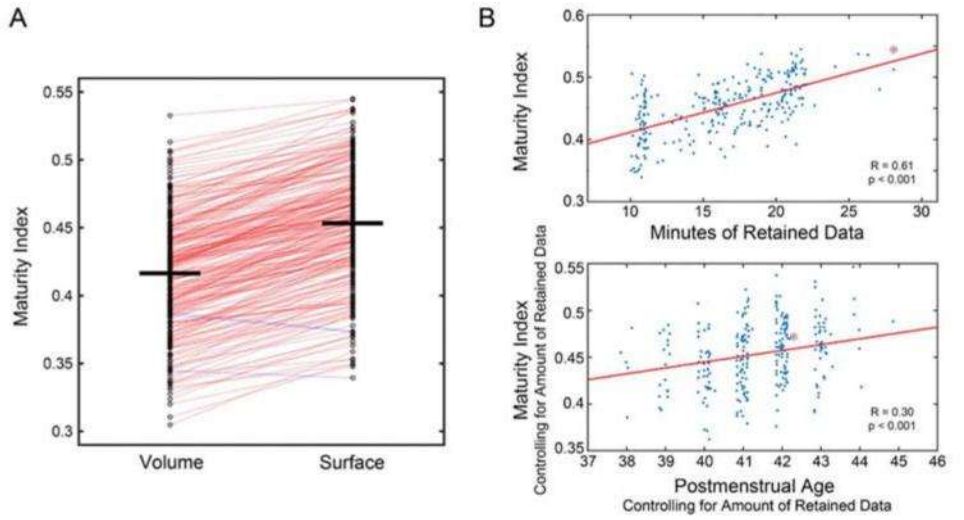


Fig. 4. Neonatal functional brain networks appear more adult-like when using surface-based alignment and as per-participant data quantity increases. **Panel A** illustrates the apparent maturity of neonatal functional connectivity depending on whether surface-based or volume-based alignment strategies were used. Lines connect MI values for individual neonates using volume-based versus surface-based alignment. Out of 262 neonates, the MI value was higher in 259 with surface-based registration (red lines); just 3 had higher MI values with volume-based registration (blue lines). The top section of **Panel B** provides a scatterplot of the significant relation between the MI (computed in surface-space) and amount of retained imaging data in the neonatal dataset. The red circle indicates a participant who was an outlier for minutes of retained data (42 min), and so their value was Winsorized (to 28 min). The bottom section demonstrates the significant relation between PMA at scan and MI, when accounting for amount of retained data.

Other groups have previously come to similar conclusions, finding that surface-based methods have better spatial accuracy and sensitivity for localizing the BOLD signal[5–7]. Volume-based analyses result in greater signal degradation and blurring, which may have strong effects on the graph-theoretic analyses we use here, due to the explicit analysis of connectivity between neighboring regions that may be artificially inflated by volume-based methods[7]. For this reason, we deferred undertaking these analyses in volume space.

6. Given the description that 261 total subjects were included in the analysis and 261 subjects were included in the neonatal timepoint, there should be no subjects starting at 2 or 3 years of age in their trajectory plots in all figures but this seems not to be the case. Can the authors clarify? A more detailed description of the distribution of the longitudinal timepoints for the 261 subjects (e.g., how many with all 0-2-3, how many have 0-2, 2-3, 0-3, or cross-sectional data only) would help readers better understand the dataset.

This is an important point, and we apologize for the oversight. In addition to the aforementioned issues with bringing participants back at later timepoints during COVID, our high standards for fMRI data inclusion (*vis-a-vis* motion censoring and amount of data retained) meant that some participants had timepoints dropped due to insufficient data quality. Participants who have data starting at years 2 or 3 did attend the neonatal scan timepoint, but their fMRI data at the neonatal timepoint was not high-quality enough to be eligible for inclusion in these analyses (see ***Participants*** section of ***Methods***).

However, we agree that characterizing this aspect of the cohort would be useful. We have added ***Supplementary Table 4***, which covers this information in more detail:

Supplementary Table 4. Sample sizes for each set of timepoints

Birth only	Y2 only	Y3 only	Birth-Y2	Birth-Y3	Birth-Y2-Y3	Y2-Y3
160	13	3	42	26	33	4

Reviewer #2 (Remarks to the Author):

This manuscript examines the development of intrinsic cortical brain networks during the first three years of life and how features of the early environment (SES) affect brain functional development. The study finds that cortical network segregation (which has been found to be associated with cognitive abilities in adolescents and adults) increases from birth to age three, with the most significant development occurring during the first two years. Prenatal SES was found to moderate the trajectory of cortical network segregation, with children from lower SES backgrounds demonstrating faster increases in segregation. These effects were most pronounced in the somatomotor and dorsal attention systems. Moreover, this study links differences in local segregation at two years of age with language and cognitive abilities, underscoring important links between early brain network development and later cognitive outcomes.

Overall, this manuscript was exceptional. The studies were elegantly designed, the research questions and analytic approaches were appropriately (and strongly) justified, and the statistical analyses were sophisticated and clean. Moreover, the paper itself was written in a clear and understandable manner. I particularly appreciated the authors' efforts to enhance transparency and reproducibility (e.g., preregistration, making code available, and excellent detail in the description of methods.). I wish every paper I reviewed was as solid as this one!

Thank you, this feedback was very nice to read! We appreciate the reviewer's detailed reading and their positive appraisal of our manuscript.

My sole comment is that I would have liked to see information provided about the race/ethnicity of the children. I think this information could help in future work seeking to extend/generalize the findings and help identify potential culturally specific relationships.

We agree with the reviewer that knowing the race/ethnicity of the sample would contextualize our findings better, and apologize for the oversight. We have added children's race and ethnicity to ***Supplementary Table 1***, which we have copied below for ease of review.

Supplementary Table 1. Participant demographics at birth.

Variable	**N = 261**[1]
Age at scan (months)	41.3 (38.0 - 45.0)
Child race	
Black	156 / 261 (60%)
Chinese	2 / 261 (0.8%)
Multiracial	3 / 261 (1.1%)
Other	1 / 261 (0.4%)
Other Pacific Islander	1 / 261 (0.4%)
White	98 / 261 (38%)
Child ethnicity	
Not Hispanic or Latino	253 / 261 (97%)
Hispanic or Latino	6 / 261 (2.3%)
Unspecified	2 / 261 (0.8%)
Child sex	
Male	141 / 261 (54%)
Female	120 / 261 (46%)
Gestational age (weeks)	38.9 (37.0 - 41.6)
Birthweight (g)	3,274.0 (2,200.0 - 4,627.0)
Area Deprivation Index	67.5 (6.0 - 100.0)
Income to Needs Ratio	2.7 (0.4 - 9.4)
Highest level of parent education completed	
Less than 12th grade	22 / 251 (8.8%)
High school degree/GED	101 / 251 (40%)
Some college/vocational school	44 / 251 (18%)
College degree (4 years)	29 / 251 (12%)
Graduate degree	55 / 251 (22%)
Insurance status (private)	135 / 261 (52%)

Healthy Eating Index	58.7 (33.0 - 80.7)
Socioeconomic disadvantage factor score	-0.1 (-2.2 - 1.5)

[f]Mean (Range); n / N (%)

Reviewer #3 (Remarks to the Author):

This study investigated the role of prenatal socioeconomic disadvantage on the pace of cortical network development through age 3. The findings suggest that cortical network segregation increases with age, and that those from lower-SES backgrounds showed faster increases relative to those from higher-SES backgrounds. The study has several strengths, including a longitudinal sample with pre- and post-natal measures, examination of non-linear associations, and preregistered analyses. I have the following suggestions and points of clarification:

We thank the reviewer for their careful reading and positive assessment of our manuscript, as well as their helpful suggestions, which have improved the clarity of the work.

Overall, I think that the study would be clearer and more impactful with more specific framing of the construct of socioeconomic status. Variations in terminology and operationalization are a persistent challenge in this area of work, and the paper would benefit from greater clarity in this area. A clear definition of SES in the introduction would help. It seems that most of the prior work that is reviewed focuses on income, education, or neighborhood SES. However, the SES variable in the current study also includes maternal nutrition and insurance status. Is this perhaps capturing a broader construct of social disadvantage? Is maternal nutrition best treated as an indicator of social and economic status, or is it something that is predicted by social and economic status?

That said, I do appreciate the sensitivity analyses that demonstrate that the results are consistent even when SES is operationalized as just income and education.

We thank the reviewer for raising this important point. We chose to label the social disadvantage construct as SES for accessibility and consistency with the broader body of research on early environments and brain development. Taking our range of sensitivity analyses into account, we concluded that the associations we find with cortical network segregation are driven primarily by socioeconomic factors captured in the social disadvantage variable.

However, we agree that social disadvantage is a broader construct that encompasses both the measures of SES the reviewer mentions (income, education, neighborhood advantage) and the inclusion of factors like maternal nutrition. We have changed our wording through the manuscript from “SES” to “disadvantage,” as shown in the example sentence below:

We observed significant and similar patterns of interactions between prenatal disadvantage and age across multiple scales, such that infants and toddlers from more disadvantaged backgrounds show a faster increase in cortical network segregation than infants and toddlers from less disadvantaged backgrounds, ending up at a higher level of network segregation.

We have also included a sentence in the *Introduction* contextualizing our use of the term "disadvantage," copied below for ease of review.

One environmental factor that is associated with the development of cortical network segregation — as well as many later life outcomes, including physical wellbeing[13,14], cognitive ability[15], and mental health[16] — is social disadvantage. Disadvantage is a broad and multifaceted construct that encompasses measures of socioeconomic status (SES) such as income, education, and occupational prestige[17,18], as well as related health factors such as insurance and diet quality[19].

There seems to be a large amount of attrition over time, with 261 families at the first time point, but only 66 at the third. It is clear why some participants were omitted due to exclusion criteria, but not how many participants dropped out between time points and why. In the results, there is mention of COVID-related challenges—did the authors examine whether excluded participants were significantly different from included participants in any way?

This is an important point, and we thank the reviewer for raising it. To clarify, the SARS-CoV-2 pandemic occurred during data collection for our two-year time point, precluding data collection from many of our participants during that time period. While the original study design was only to collect data at two years of age, due to the large amount of data collection missed, we then tried to collect data again at three years of age. We had 202 participants (of the original 385 participants with scans) return at the two-year follow-up, and 132 (of the original 385) at the three-year follow-up. Missing participants to follow-up occurred in large part, as the reviewer notes, because of COVID-related restrictions on scanning. After exclusions for imaging quality and health, we have a final sample size of n = 92 at the two-year time point and n = 66 at the three-year time point.

We now perform additional analyses examining whether the participants who did not return for later visits are significantly different on key demographic variables than those who did. We include these findings in the ***Methods*** on page 22:

Participants who returned for data collection at year two were not significantly different than participants from whom we were not able to collect year two data in gestational age, birthweight, disadvantage, psychosocial stress, sex, neighborhood deprivation, or income-to-needs ratio (two-sided t-test, all p's > 0.05). Participants who returned for data collection at year three were significantly more advantaged (lower prenatal social disadvantage, $t(358) = -2.42$, $p = 0.016$) than participants from whom we were not able to collect year three data; there were no other significant differences between groups (p's > 0.05).

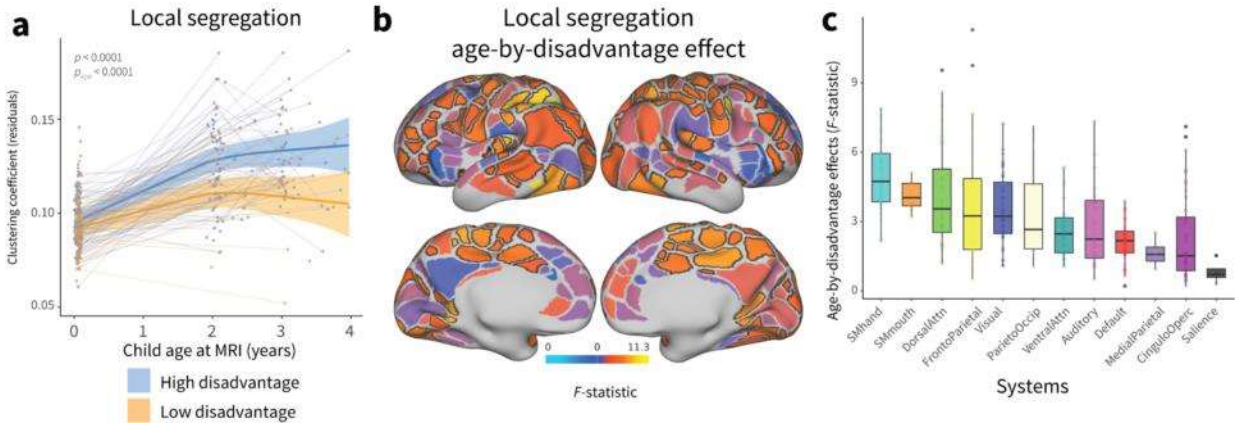
We too were concerned about attrition, and so we conducted a supplemental analysis examining whether our findings held when only examining participants who had longitudinal data, ensuring consistency of demographic variables across time points. In ***Supplementary Figure 1***, you will find that although we have a significant decrease in sample size, we find similar patterns to those reported in the main text.

Relatedly, were any power analyses conducted?

This is a good question. These analyses were conceptualized and pre-registered during collection of the three-year data, and thus we used all the available neuroimaging data as part of this longitudinal sample. Our sample size was not chosen based on a pre-specified power analysis. Additionally, conducting power analyses in the GAMM framework is a non-trivial endeavor, as we must specify the predicted shape (number of basis functions) of the smooth; since few studies exist in our age range to provide estimates of this, we chose to simply pre-register our model specifications.

The figures (e.g., Fig 1) seem to depict some potential outliers. For example, there is an average increase in segregation, but some children show sharp decreases in segregation, and others have especially high segregation. Were there any significant outliers? The preregistration mentions examination of outliers, but I do not think this was described in the manuscript.

We thank the reviewer for raising this important point, and apologize for the oversight. Regarding outliers, in our pre-registration, we proposed several sensitivity analyses to ensure that our results were robust to methodological variation and choices about data inclusion. One of these analyses entailed excluding observations where measures of network segregation are > 3 SD away from the mean. Our results are qualitatively (and quantitatively) similar when excluding those observations. We now include this in the supplement, in ***Supplementary Figure 5***:



Supplementary Figure 5. Associations between the early environment and developmental increases in cortical network segregation, excluding outliers > 3 SD away from the mean. **a,** Prenatal disadvantage moderates trajectories of local cortical network segregation. **b,** The heterogenous patterning of the magnitude of age-by-disadvantage effects (*F*-statistic) on local segregation is shown on the cortical surface. Regions that show significant age-by-disadvantage effects passing FDR correction at $p_{FDR} < 0.05$ are outlined in black. **c,** Disadvantage effects on developmental increases in local segregation are enriched in sensorimotor systems. Boxplots show median and interquartile range of the magnitude of age-by-disadvantage effects; each point is an individual parcel.

We excluded outlier observations, that is, observations at a timepoint that were > 3 SD away from the mean, and find that our results are qualitatively similar. Global segregation ($F_{s(agexSES)} = 2.91$, $p = 0.05$, $p_{FDR} = 0.069$), meso-scale segregation ($F_{s(agexSES)} = 7.68$, $p = 0.0005$, $p_{FDR} = 0.001$), and local segregation (***Supplementary Figure 5a,*** $F_{s(agexSES)} = 13.32$, $p < 0.0001$, $p_{FDR} < 0.0001$) show similar patterns of interactions, such that infants and toddlers from more disadvantaged backgrounds show a faster increase

in cortical network segregation than infants and toddlers from less disadvantaged backgrounds. We found no evidence for moderating effects of disadvantage on developmental changes in network integration ($F_{s(agexSES)} = 1.862$, $p = 0.16$, $p_{FDR} = 0.16$). The magnitude of disadvantage effects on developmental increases in local segregation differed across functional systems, with the strongest effects found in somatomotor-hand, somatomotor-mouth, dorsal attention, and frontoparietal systems (***Supplementary Figure 5c***).

What are the implications of applying adult atlases and cortical networks to infant data? This is a potential limitation that could be mentioned.

We agree with the reviewer that our interpretations are limited by the use of adult systems and parcellations. We have now added this as a limitation in the ***Discussion***, on page 16:

Third, we employed a common adult parcellation and set of systems to characterize cortical network development. It is possible that differences in the degree to which a neonate's fine-grained cortical topography resembles that of an adult might influence measures of cortical network segregation; the continued development of neonatal parcellations[93,94] will enable future work to use infant-specific parcellations and systems to characterize brain development.

Greater attention to the underlying mechanisms would strengthen the paper. What is it about low SES that is hypothesized to alter the tempo of cortical development? This is alluded to in the introduction, but could be more explicitly laid out. For example, are the authors conceptualizing low SES as a "harsh and unpredictable environment" or an indication of deprivation/inadequate care?

The reviewer makes an important point that the underlying mechanisms are fundamentally the factor of most interest. We have added a sentence in the ***Introduction*** to touch upon a framework that we have previously proposed, and more importantly, now discuss this in the ***Discussion*** in a separate paragraph, copied below for ease of review.

There are several possible mechanisms by which variation in the early environment could signal to alter the tempo of brain development. One commonly proposed mechanism is deprivation, where lack of expected inputs at a developmental stage results in earlier pruning of synapses and reduced synaptic connectivity[67,68]. Recently, we put forth a model in which the valence and variability of experiences interact to predict maturational pace, such that experiences that are chronic and negative encourage faster maturation and restrict plasticity, and experiences that are novel and positive delay maturational processes and enhance plasticity[22]. Growing up in a more advantaged environment is associated with more cognitively enriching, positively valenced experiences[69]. This environment — the opposite of deprivation[67] — may delay maturational processes and prolong plasticity: animals exposed to enriched environments as juveniles display enhanced markers of synaptic and extracellular markers of plasticity[70,71], and the release of neurotransmitters associated with positive experiences increases cortical plasticity and facilitates remodeling[72–74]. Conversely, negative experiences such as stress might accelerate brain development through several different mechanisms[75–77]; higher disadvantage is consistently associated with higher stress[78]. Although in this work we find that prenatal socioeconomic disadvantage has stronger effects on brain development than does psychosocial stress, our measurement of stress was

limited; stress may still be one of several mechanisms by which disadvantage results in changes in the tempo of brain development.

The variability of experiences might also interact with the valence of experiences to predict maturational pace. Repeated exposure to the same experience, signaling to the brain to optimize for the continued occurrence of this experience in the future, can accelerate maturation of specific circuitry [79–81]. This aligns with theories of stress effects on maturational pace, where repeated stress-detection and stress-regulation leads to faster maturation of the amygdala and medial prefrontal cortex[75,82]. In contrast, rare or highly variable experiences could signal that the environment is still changing, and that plasticity is beneficial[83]. Computational evolutionary models suggest that individuals who have more variable experiences lose plasticity later in development than those with more consistent experiences[84–86], and some evidence from the study of critical periods suggests that periods of plasticity are prolonged when environmental statistics are variable or unreliable[87,88]. Further work testing these models and delineating which dimensions are most important contributors to the observed effects of the early environment on the pace of brain development will be key to understanding the mechanisms by which environmental cues give rise to changes in maturational tempo.

The final sentence of the manuscript is quite vague and thus does not add much to the paper: "Our results suggest that infancy and toddlerhood may be an important period for promoting healthy brain development and emphasize the first years of life as a target for policies aimed at supporting optimal child development." If the authors wish to comment on policy implications, I think this could be done in a more thorough and specific way that is more directly linked to the data in the current study.

We appreciate the reviewer's focus on clarity. We have revised this sentence to read as follows:

Our results emphasize the importance of expanding and enhancing policies that provide financial support to parents of young children[96–99], and further underlines the role of anti-poverty initiatives in promoting children's healthy brain development.

References

1. Luby, J. L. *et al.* Social disadvantage during pregnancy: effects on gestational age and birthweight. *J Perinatol* **43**, 477–483 (2023).
2. MacCallum, R. C., Zhang, S., Preacher, K. J. & Rucker, D. D. On the practice of dichotomization of quantitative variables. *Psychological Methods* **7**, 19–40 (2002).
3. Thoresen, M. Spurious interaction as a result of categorization. *BMC Medical Research Methodology* **19**, 28 (2019).
4. Sylvester, C. M. *et al.* Network-specific selectivity of functional connections in the neonatal brain. *Cerebral Cortex* bhac202 (2022) doi:10.1093/cercor/bhac202.
5. Jo, H. J. *et al.* Spatial accuracy of fMRI activation influenced by volume- and surface-based spatial smoothing techniques. *NeuroImage* **34**, 550–564 (2007).
6. Andrade, A. *et al.* Detection of fMRI activation using Cortical Surface Mapping. *Human Brain Mapping* **12**, 79–93 (2001).
7. Brodoehl, S., Gaser, C., Dahnke, R., Witte, O. W. & Klingner, C. M. Surface-based analysis increases the specificity of cortical activation patterns and connectivity results. *Sci Rep* **10**, 5737 (2020).
8. Kachmar, A. G., Connolly, C. A., Wolf, S. & Curley, M. A. Q. Socioeconomic Status in Pediatric Health Research: A Scoping Review. *The Journal of Pediatrics* **213**, 163–170 (2019).
9. Ramphal, B. *et al.* Brain Connectivity and Socioeconomic Status at Birth and Externalizing Symptoms at Age 2 Years. *Developmental Cognitive Neuroscience* 100811 (2020) doi:10.1016/j.dcn.2020.100811.
10. Marcin, J. P., Schembri, M. S., He, J. & Romano, P. S. A Population-Based Analysis of Socioeconomic Status and Insurance Status and Their Relationship With Pediatric Trauma Hospitalization and Mortality Rates. *Am J Public Health* **93**, 461–466 (2003).

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have fully addressed all my concerns.

Reviewer #2 (Remarks to the Author):

The authors have thoughtfully addressed all of my concerns.

Reviewer #3 (Remarks to the Author):

I appreciate the authors' careful and rigorous work on this revision. I found all of the reviewer comments were thoroughly addressed and have greatly strengthened the manuscript. This paper will be an excellent contribution to the field.