Supplementary material to

# Maternal psychological distress associates with alterations in resting-state low-frequency fluctuations and distal functional connectivity of the neonate medial prefrontal cortex

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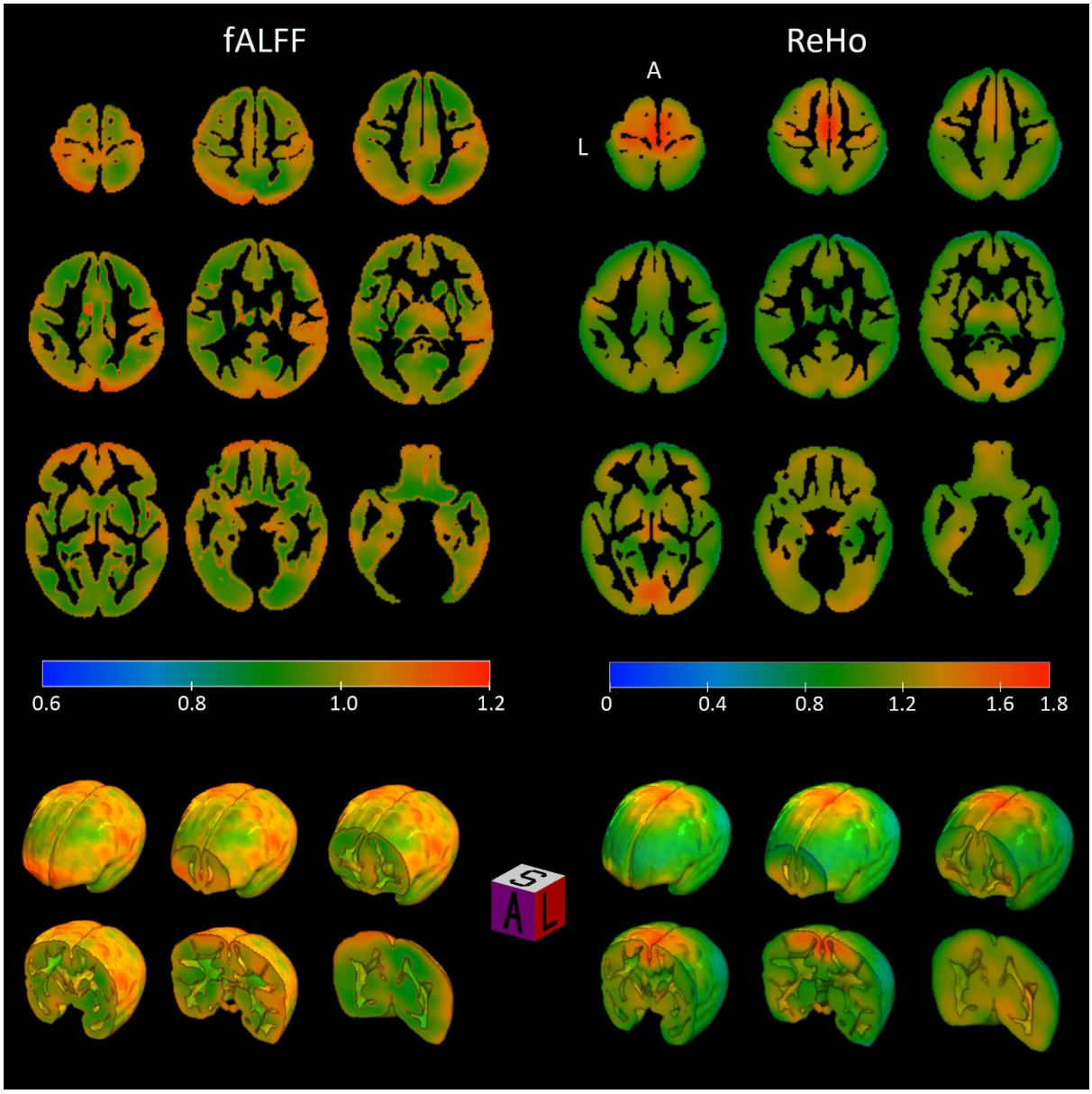
The purpose of this supplementary material is to provide the reader with:

1. Mean fALFF and ReHo maps of the neonate brain at 26.14 ± 6.28 days after birth
2. Effects of additional independent variables (IV) on primary models (sensitivity analyses):
    a. Neonate birth weight
    b. Maternal age
3. Exclusion/subgroup analysis without subjects with exposure to illicit substances/alcohol (N=18)
    a. Composite score model
4. Non-parametric composite score model results
5. Correlation matrix of measures used in this study
6. Cluster coordinates and effect sizes of parametric, non-parametric composite score and sensitivity analysis results
7. One sample T-test of mPFC seed connectivity
8. Group-level multiple regression results of PSE on neonate mPFC FC at a more stringent threshold
9. Estimated motion parameters table
10. Parametric fALFF results of SCL and EPDS score models

## 1. Mean fALFF and ReHo maps (N=21) of the neonate brain at 26.14 ± 6.28 days after birth.




**Supplementary materials, Figure 1**. Mean fALFF and ReHo maps of the neonate brain displayed on axial slices. Color bar denotes mean fALFF values. High mean fALFF values are located on the sensorimotor, parietal, temporal, visual, anterior prefrontal and basal ganglia regions. Mild asymmetry can be observed in temporal cortex and basal ganglia. Similarly high fALFF values in sensorimotor, visual and medial prefrontal regions have been previously reported in a larger sample (1).

## 2. Effects of additional independent variables on primary models (sensitivity analyses):

### a. Composite score and neonate birth weight

In this multiple regression model, neonate birth weight was set as a 4$^{th}$ independent variable (IV) of no interest. Otherwise, we used prior default IVs: Neonate age at scanning (days), neonate sex and maternal pre-pregnancy BMI. The complete model thus consisted of the beforementioned IVs and composite score as the main explanatory variable (EV).

In this model, the effect observed in our main analysis of maternal composite score on neonate fALFF maps was reduced to statistical insignificance at $p < 0.001$ and $p < 0.005$ levels. No clusters that passed the multiple comparison statistical thresholding were detected.

Bivariate correlation analysis performed in SPSS revealed a significant correlation between neonate birth weight and composite score ($r_s = -0.606$). Variance of inflation (VIF) analysis revealed no indications of multicollinearity (VIF = 1.479). Even though there is an established link between neonate birth weight and exposure to prenatal stress (1), there is little reason to believe that infant birth weight itself would be the driving factor for effects seen in neonate fALFF maps. Nevertheless, to test this possibility we generated an additional model with infant birth weight as the EV and with neonate age at scanning, sex, and maternal pre-pregnancy BMI as the IVs. No statistically significant effects at $p < 0.001$ or $p < 0.005$ FWE-corrected level were obtained in this model.

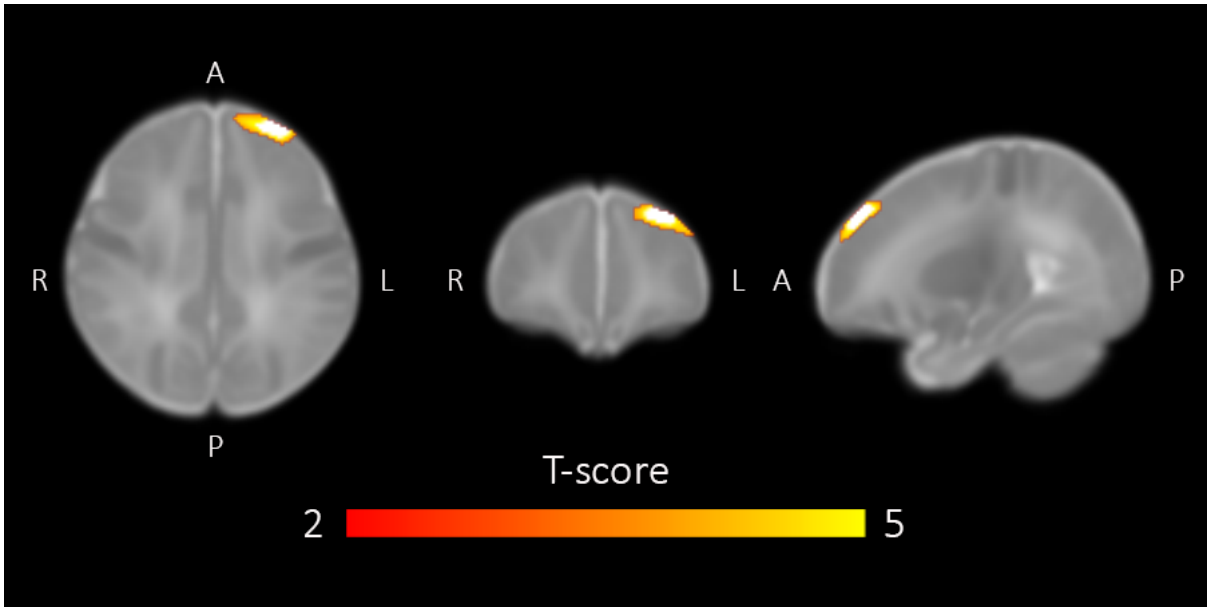
We conclude that the mitigation of results in our main model, when corrected for neonate birth weight, was caused by the high negative correlation between neonate birth weight and composite score.

### b. Composite score and maternal age in years

Here, the multiple regression design consisted of four IVs of no interest: Neonate age at scanning (days), neonate sex, maternal pre-pregnancy BMI and maternal age in years. Composite score was set as the main EV.

The effects were reduced to statistical insignificance at $p < 0.001$ and $p < 0.005$ levels. The performed bivariate correlation analysis revealed that maternal age in years had a significant correlation with maternal pre-pregnancy BMI ($r_s = 0.570$), but no significant correlation was observed between composite score and maternal age. VIF analysis showed no indications of multicollinearity (VIF = 1.549) in this model.

Maternal age has been established to associate with levels of mental distress during pregnancy (2). To test whether maternal age had an independent effect on neonate fALFF maps, we performed another analysis with neonate age at scanning, neonate sex and maternal pre-pregnancy BMI as IVs. In this model, maternal age was set as the main EV. Here, we found a statistically significant (at $p < 0.001$ level) effect localizing to the left superior frontal gyrus ($p < 0.001$ FWE-corrected, cluster size of 903 voxels). The results are displayed in Supplementary materials figure 2.




**Supplementary materials, figure 2.** Regions where fALFF significantly correlated with maternal age in years ($p < 0.001$ FWE-corrected) in the naturally sleeping neonate (N = 21). Highlighted region entails the left superior frontal gyrus. Color bar represents T-scores. Images are displayed in radiological convention on the UNC neonate template in axial, coronal and sagittal slices. Abbreviations: A = Anterior, P = Posterior, L = Left, R = Right.

Out of the five papers (3–7) investigating PSE effects on neonate rs-fMRI metrics, only one controlled for maternal age at beginning of pregnancy (4), with similar maternal age distribution as in our sample. They found no association between maternal age and neonate FC maps. Considering extant literature and the results of these sensitivity analyses, we cannot rule out that the effects of maternal psychological distress on offspring brain development might depend on maternal age.

### 3. Exclusion analysis (N=18)

To make sure the results of our main model were not influenced by exposure to illicit substances and/or alcohol, we performed an additional analysis, in which the exposed subjects were excluded.

#### a. Composite score model

In this model, we excluded the three subjects that were exposed to illicit substances (cannabis) and/or alcohol *in utero*, yielding a sample size of 18 subjects. Otherwise, identical design was used as in the main parametric model with neonate age at scanning, neonate sex and maternal pre-pregnancy BMI set as IVs. The composite score was set as the main EV. Statistical significance threshold was set to $p < 0.001$.

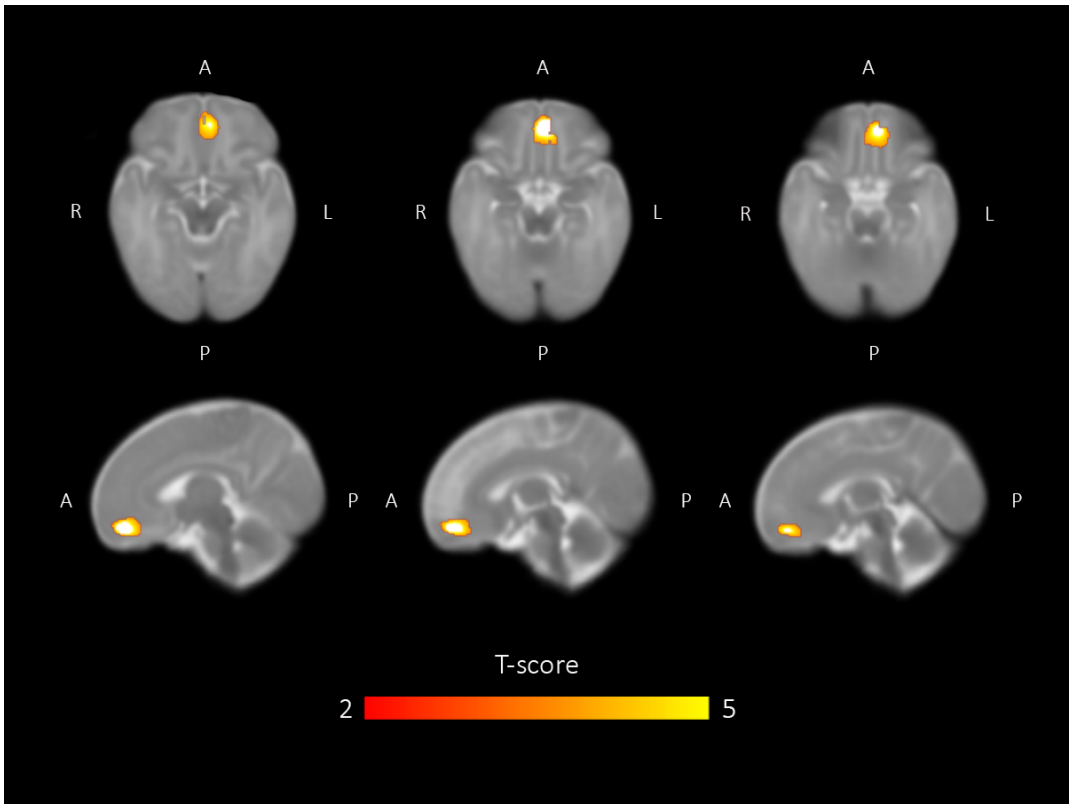
We obtained near-identical results with the exclusion analysis as with our main model with 21 subjects. A statistically significant effect was observed in the neonate mPFC ($p < 0.001$ uncorrected; $p < 0.001$ FWE-corrected; kE 796). Here, the cluster shape was slightly altered (supplementary materials, figure 2) and fragmented into two separate clusters.

Although minimally altered, this model yielded a highly comparable result to our main model with 21 subjects. In all cases, exposure to alcohol and/or illicit substances was mild. After consideration, we decided to include the three subjects exposed to alcohol for increased statistical power in our main model.

## 4. Non-parametric main model results (SnPM13)

To test the validity of the underlying assumptions in the main parametric model, we repeated the analysis using non-parametric permutation testing with the Statistical Non-Parametric Mapping software (SnPM13). As the parametric model, the non-parametric model had the following measures set as IVs: neonate age at scanning, neonate sex and maternal pre-pregnancy BMI. Composite score was set as the main EV. Statistical significance threshold was set to $p < 0.001$.

We found identical results as with the parametric model. The composite score – fALFF effect localized to the neonate mPFC ($p < 0.001$ uncorrected; $p < 0.001$ FWE-corrected; kE 794). No additional statistically significant clusters nor negative associations were observed.




**Supplementary materials, figure 3.** Regions where fALFF significantly correlated with maternal composite score ($p < 0.001$ FWE-corrected) in the naturally sleeping neonate (N = 21) with non-parametric complementary model. Highlighted region entails the ventromedial prefrontal cortex. Color bar represents T-scores. Images are displayed in radiological convention on the UNC neonate template in axial and sagittal slices. Abbreviations: A = Anterior, P = Posterior, L = Left, R = Right.

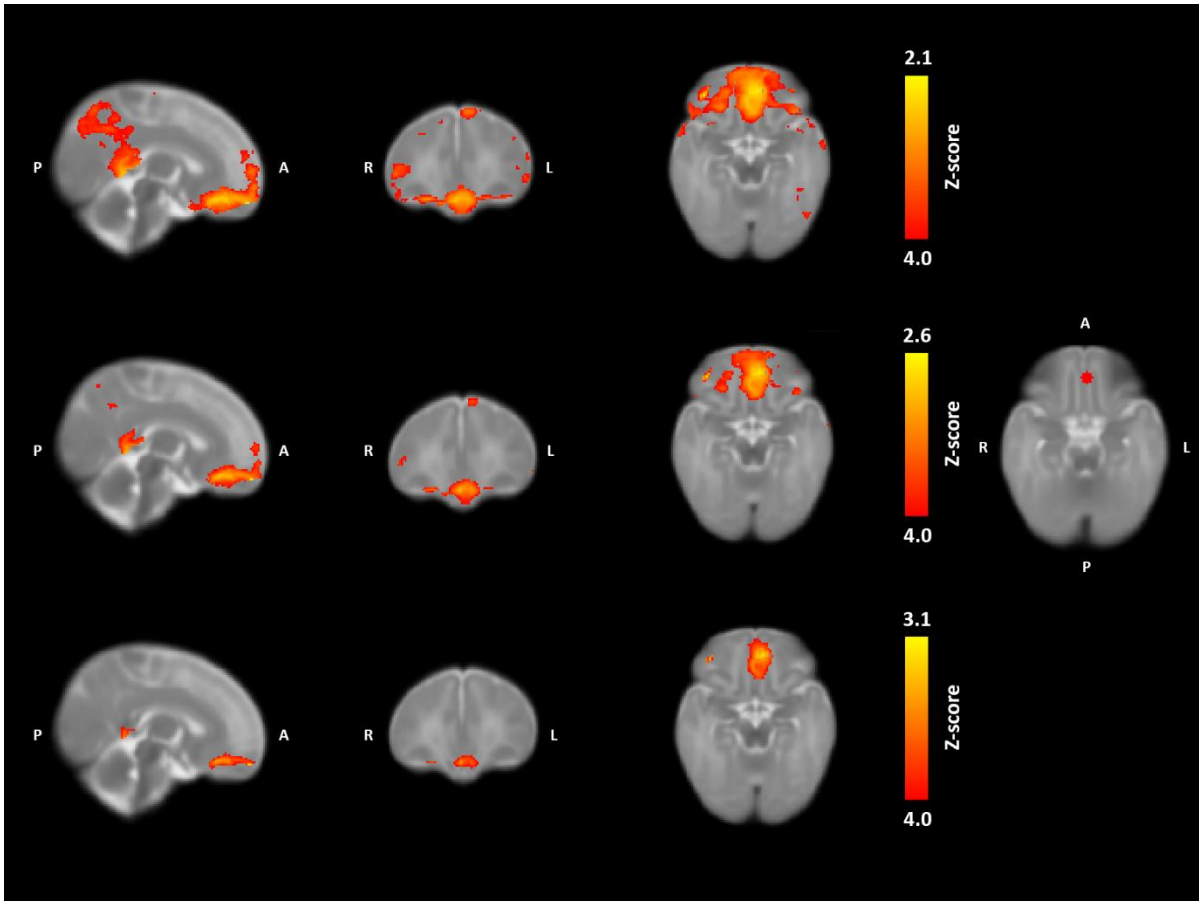
5. **Supplementary materials, table 1.** Correlation matrix for metrics involved in this study. $r_s$ = Spearman rank correlation coefficient. * and ** denote statistically significant correlation at $p < 0.05$ and $p < 0.01$ levels, respectively.

| | Maternal age in years | Neonate birth weight | Maternal pre-pregnancy BMI | SCL-90 | EPDS | Composite score |
|---|---|---|---|---|---|---|
| Maternal age in years | $r_s = 1.000$<br>p = N/A | $r_s = -0.038$<br>p = 0.871 | $r_s = 0.570$**<br>p = 0.007 | $r_s = -0.193$<br>p = 0.402 | $r_s = -0.112$<br>p = 0.629 | $r_s = -0.126$<br>p = 0.586 |
| Neonate birth weight | $r_s = -0.038$<br>p = 0.871 | $r_s = 1.000$<br>p = N/A | $r_s = 0.200$<br>p = 0.385 | $r_s = -0.447$<br>p = 0.042 | $r_s = -0.675$**<br>p = 0.001 | $r_s = -0.606$**<br>p = 0.004 |
| Maternal pre-pregnancy BMI | $r_s = 0.570$**<br>p = 0.007 | $r_s = 0.200$<br>p = 0.385 | $r_s = 1.000$<br>p = N/A | $r_s = -0.500$*<br>p = 0.021 | $r_s = -0.342$<br>p = 0.129 | $r_s = -0.465$*<br>p = 0.033 |
| SCL-90 | $r_s = -0.193$<br>p = 0.402 | $r_s = -0.447$<br>p = 0.042 | $r_s = -0.500$*<br>p = 0.021 | $r_s = 1.000$<br>p = N/A | $r_s = 0.599$**<br>p = 0.004 | $r_s = 0.845$**<br>p = 0.000 |
| EPDS | $r_s = -0.112$<br>p = 0.629 | $r_s = -0.675$**<br>p = 0.001 | $r_s = -0.342$<br>p = 0.129 | $r_s = 0.599$**<br>p = 0.004 | $r_s = 1.000$<br>p = N/A | $r_s = 0.911$**<br>p = 0.000 |
| Composite score | $r_s = -0.126$<br>p = 0.586 | $r_s = -0.606$**<br>p = 0.004 | $r_s = -0.465$*<br>p = 0.033 | $r_s = 0.845$**<br>p = 0.000 | $r_s = 0.911$**<br>p = 0.000 | $r_s = 1.000$<br>p = N/A |

6. **Supplementary materials, table 2.** Model results with cluster coordinates, sizes and locations. Cluster coordinates are defined in the UNC neonate template (MNI).

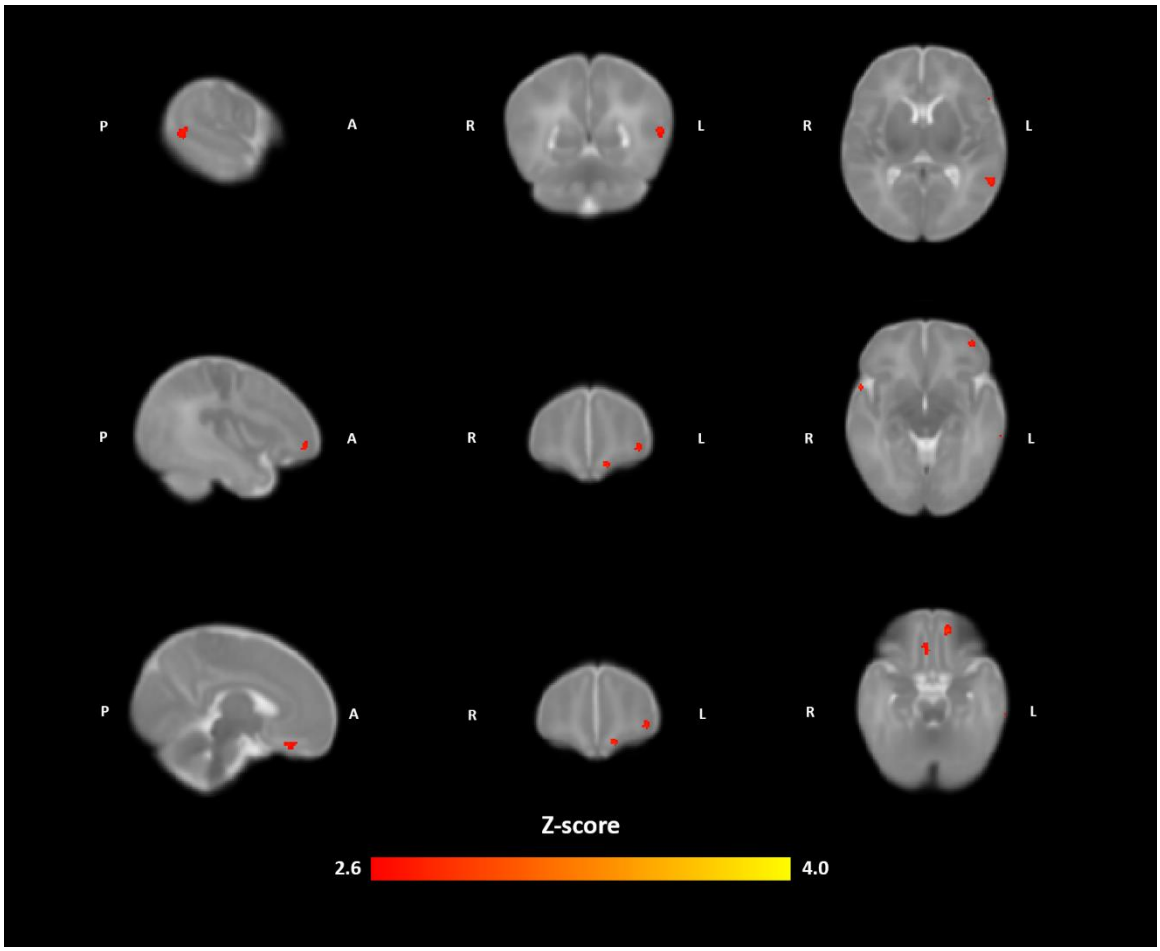
| **Model** (**EV** and IVs) | **p-threshold** | **p (FWE-corrected)** | **Cluster size in voxels (kE)** | **Cluster peak coordinates (X, Y, Z)** |
|---|---|---|---|---|
| • **Composite score**<br>• Neonate age at scanning<br>• Neonate sex<br>• Maternal pre-pregnancy BMI | p < 0.001 | p < 0.001 | 794 | 2, 44, -29 |
| **Non-parametric designs of the original model (SnPM13)** | | | | |
| • **Composite score**<br>• Neonate age at scanning<br>• Neonate sex<br>• Maternal pre-pregnancy BMI | p < 0.001 | p < 0.001 | 794 | 2, 44, -29 |
| **Additional models (sensitivity analyses)** | | | | |
| • **Composite score**<br>• Neonate age at scanning<br>• Neonate sex<br>• Maternal pre-pregnancy BMI<br>• Neonate birth weight | p < 0.01 | p < 0.007 | 1601 | 2, 44, -29<br>3, 53, -19<br>10, 36, -30 |
| • **Composite score**<br>• Neonate age at scanning<br>• Neonate sex<br>• Maternal pre-pregnancy BMI<br>• Maternal age | p < 0.05 | p < 0.023 | 5418 | 1, 44, -29<br>1, 53, -22<br>3, 29, -31 |

## 7. One sample T-test of mPFC seed connectivity




**Supplementary materials, figure 4.** One-sample T-test results displaying mean connectivity from seed ROI (mPFC) in the neonate brain (N = 21). Horizontal rows correspond to connectivity patterns with distinct significance thresholds (with Z-score 2.1 corresponding to $p < 0.05$; Z-score 2.6 to $p < 0.005$; Z-score 3.1 to $p < 0.001$; all multiple comparison corrected at cluster-level). Seed ROI (x = 88, y = 148, z = 59 in the UNC template space) is illustrated on the right-side brain image. Color bar represents Z-scores. Images are displayed in radiological convention on the UNC neonate template. Abbreviations: mPFC = medial prefrontal cortex; ROI = region-of-interest, A = Anterior, P = Posterior, R = Right, L = Left.

## 8. Group-level multiple regression results of PSE on neonate mPFC FC at more stringent threshold.



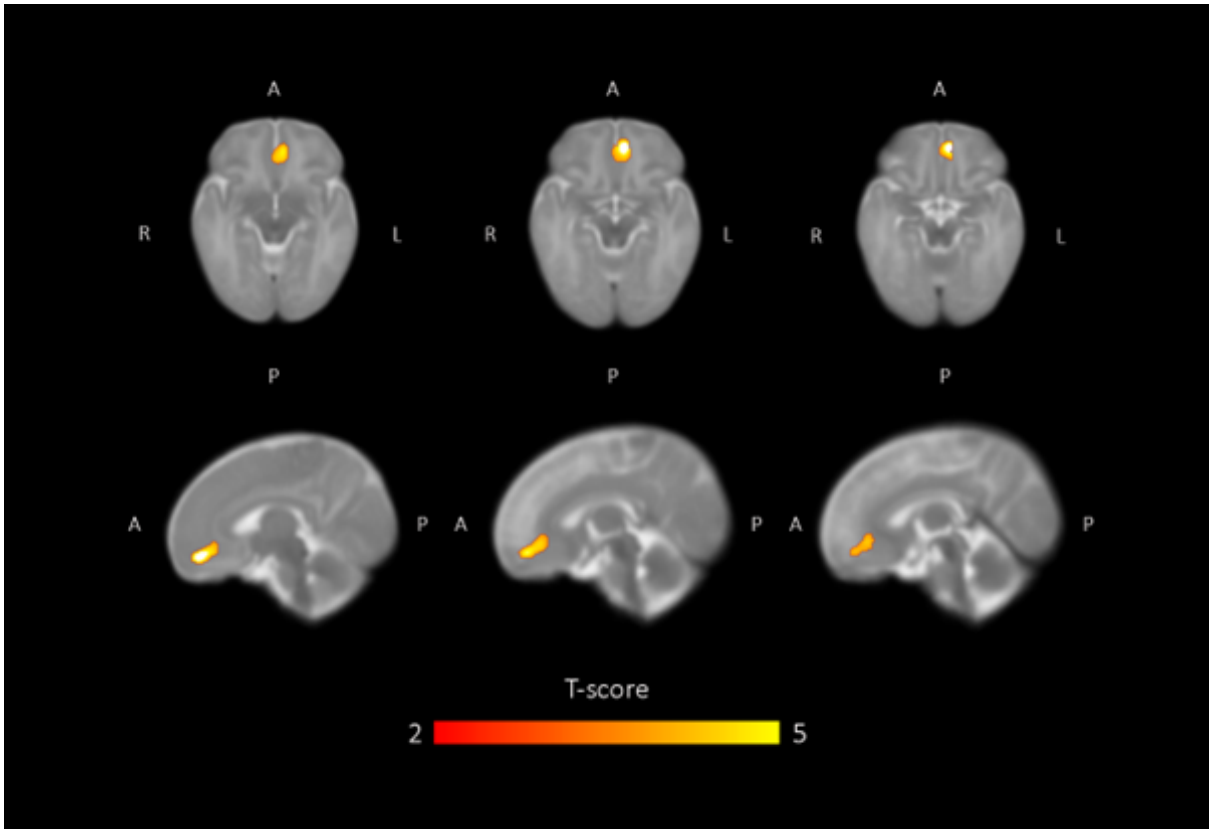

**Supplementary materials, figure 5.** Brain regions where maternal composite score was positively correlated (Z-score threshold of 2.6, corresponding to $p < 0.005$ multiple comparison corrected at cluster-level) with neonate mPFC seed connectivity in multiple regression analysis (N = 21). For mPFC seed ROI definition see supplementary materials, figure 4. Images are displayed in radiological convention on the UNC neonate template. Abbrevations: mPFC = medial prefrontal cortex; ROI = region-of-interest, A = Anterior, P = Posterior, R = Right, L = Left.

## 9. Estimated motion parameters.

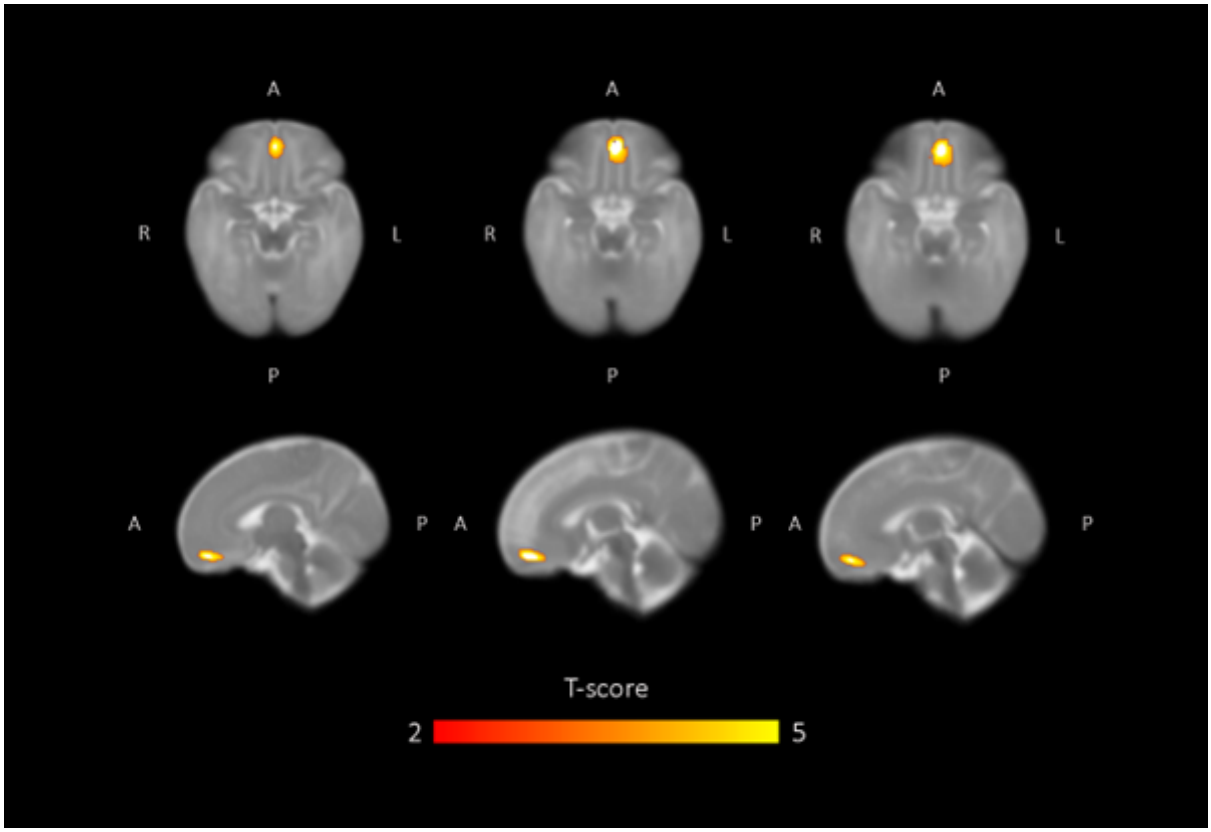
| Variable | Absolute mean | Max | Min | fALFF, Z-score $r_s$ (p-value) | SCA, Z-score $r_s$ (p-value) |
|---|---|---|---|---|---|
| Composite motion estimate (mm) | 0.53 | 1.97 | 0.05 | 0.192 (0.378) | -0.242 (0.291) |
| DVARS | 1,16 | 10,58 | 0,87 | 0.243 (0.332) | 0.185 (0.463) |
| Translation (X) (rad) | 0.13 | 0.46 | -0.12 | -0.023 (0.920) | -0.035 (0.880) |
| Translation (Y) (rad) | 0.27 | 0.07 | -2.80 | -0.234 (0.308) | -0.292 (0.199) |
| Translation (Z) (rad) | 0.55 | 0.54 | -3.13 | -0.039 (0.867) | 0.165 (0.475) |
| Rotation (X) (rad) | 0.02 | 0.07 | -0.17 | 0.073 (0.754) | -0.075 (0.746) |
| Rotation (Y) (rad) | 0.01 | 0.09 | -0.06 | -0.326 (0.149) | 0.121 (0.602) |
| Rotation (Z) (rad) | 0.04 | 6.49 | -0.00 | 0.000 (1.000) | -0.142 (0.540) |

**Supplementary materials, table 3.** Whole sample estimated motion parameters and with correlations to metric Z-scores (fALFF, SCA).

## 10. Parametric fALFF results of SCL and EPDS score models




**Supplementary materials, figure 6.** Regions where fALFF significantly correlated with maternal SCL-score ($p < 0.001$ FWE-corrected) in the naturally sleeping neonate (N = 21). Highlighted region entails the ventromedial prefrontal cortex. Color bar represents T-scores. Images are displayed in radiological convention on the UNC neonate template in axial and sagittal slices. Abbreviations: A = Anterior, P = Posterior, L = Left, R = Right.




**Supplementary materials, figure 7.** Regions where fALFF significantly correlated with maternal EPDS-score ($p < 0.001$ FDR-corrected) in the naturally sleeping neonate (N = 21). Highlighted region entails the ventromedial prefrontal cortex. Color bar represents T-scores. Images are displayed in radiological convention on the UNC neonate template in axial and sagittal slices. Abbreviations: A = Anterior, P = Posterior, L = Left, R = Right.

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