**Supplementary Materials**

**Harsh parenting, amygdala functional connectivity changes across childhood, and behavioral problems**

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**Figure S1. Sampling flowchart**

A flowchart of a flowchart

Description automatically generated

**Text S1. Details of the resting-state functional MRI preprocessing**

The rsfMRI data was preprocessed using the default MNI pipeline in the CONN toolbox (release 20b) and SPM12 in Matlab R2020a. Within each session, all volumes were co-registered and resampled to the first volume via b-spline interpolation using the SPM12 functional realignment and unwarp procedure. Temporal misalignment was corrected by using time correction with sinc interpolation in SPM12. Scan volumes with framewise displacement above 0.9mm or global BOLD signal changes above 5 standard deviations were flagged as potential outliers for confound regression. Both anatomical and functional data were normalized into standard MNI space and segmented into gray matter, white matter, and CSF tissue using SPM12 unified segmentation and normalization procedure with 4th-order spline interpolation. A 6 mm full-width half maximum (FWHM) kernel was used for Gaussian smoothing of the functional data. Denoising was performed on the data in a two-step process. First, ordinary least squares regression was used to remove potential noise confounds: cerebral blood flow, subject motion parameters, identified outliers, and constant and first-order linear session effects. Next, temporal band-pass filtering was performed to remove signals below 0.008 Hz and above 0.09 Hz. Subjects with greater than 6 mm maximum motion, 0.6 mm mean motion, or more than 20% outliers scan were excluded from the analysis. We calculated the motion of each volume using the average framewise displacement from the first volume. The maximum motion is given by the volume with the largest framewise displacement, and the mean motion was calculated by averaging the motion value across all volumes in the time series (Nieto-Castanon, 2020). The choice of 0.6mm threshold for mean motion is due to the higher amount of motion in pediatric scans. A more stringent threshold would result in lower power due to a smaller sample size. This threshold has been previously used in multiple neuroimaging studies using the same cohort (Huang et al., 2023) and similar thresholds have been used in other neuroimaging studies in children (Fan et al., 2021).

**Table S1. Comparison of sample characteristics between the original and analytical samples**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Original GUSTO cohort  (n = 1,466) | Analytical sample PSDQ-CBCL  (n = 254) | | Analytical sample PSDQ-CDI  (n = 309) | | Analytical sample PSDQ-sMRI  (n = 296) | | Analytical sample PSDQ-fMRI  (n = 256) | |
|  |  |  | P |  | P |  | P |  | P |
| Child sex, N (%) | Girl | 578 (47.5) | 113 (44.5) | 0.38 | 139 (45.0) | 0.42 | 151 (51.0) | 0.28 | 136 (53.1) | 0.10 |
|  | Boy | 638 (52.5) | 141 (55.5) |  | 170 (55.0) |  | 145 (49.0) |  | 120 (46.9) |  |
|  | Missing | 250 | 0 |  | 0 |  | 0 |  | 0 |  |
| Marital status, N (%) | Living with husband | 1,349 (95.0) | 240 (97.2) | 0.14 | 291 (97.0) | 0.14 | 281 (97.2) | 0.10 | 246 (98.0) | 0.04 |
|  | Not living with husband | 71 (5.0) | 7 (2.8) |  | 9 (3.0) |  | 8 (2.8) |  | 5 (2.0) |  |
|  | Missing | 46 | 7 |  | 9 |  | 7 |  | 5 |  |
| Maternal ethnicity, N (%) | Chinese | 823 (56.1) | 156 (61.4) | 0.03 | 178 (57.6) | 0.10 | 156 (52.7) | 0.13 | 134 (52.3) | 0.06 |
|  | Malay | 373 (25.4) | 69 (27.2) |  | 89 (28.8) |  | 92 (31.1) |  | 83 (32.4) |  |
|  | Indian | 270 (18.4) | 29 (11.4) |  | 42 (13.6) |  | 48 (16.2) |  | 39 (15.2) |  |
| Household highest education, N (%) | Primary or secondary | 109 (13.9) | 25 (12.5) | 0.61 | 34 (13.7) | 0.52 | 38 (16.5) | 0.08 | 30 (15.3) | 0.08 |
|  | ITE/MITEC | 94 (11.9) | 28 (14.0) |  | 34 (13.7) |  | 34 (14.8) |  | 31 (15.8) |  |
|  | GCE A levels/ Polytechnic/ Diploma | 204 (25.9) | 58 (29.0) |  | 73 (29.3) |  | 69 (30.0) |  | 60 (30.6) |  |
|  | University (Bachelor, Master, PhD) | 380 (48.3) | 89 (44.5) |  | 108 (43.4) |  | 89 (38.7) |  | 75 (38.3) |  |
|  | Missing | 679 | 54 |  | 60 |  | 66 |  | 60 |  |
| Household monthly income, N (%) | SGD 0 - 1,999 | 211 (15.7) | 28 (11.9) | 0.45 | 35 (12.2) | 0.48 | 42 (15.2) | 0.40 | 36 (15.1) | 0.67 |
|  | SGD 2,000 - 3,999 | 420 (31.2) | 74 (31.4) |  | 94 (32.6) |  | 95 (34.3) |  | 83 (34.9) |  |
|  | SGD 4,000 - 5,999 | 337 (25.0) | 60 (25.4) |  | 72 (25.0) |  | 75 (27.1) |  | 59 (24.8) |  |
|  | SGD ≥ 6,000 | 379 (28.1) | 74 (31.4) |  | 87 (30.2) |  | 65 (23.5) |  | 60 (25.2) |  |
|  | Missing | 119 | 18 |  | 21 |  | 19 |  | 18 |  |
| Maternal psychological symptoms, mean (SD) |  | 6.3 (7.3) | 6.7 (8.0) | 0.59 | 6.4 (7.5) | 0.85 | 6.5 (7.6) | 0.84 | 6.3 (7.8) | 0.77 |
|  | Missing | 1,021 | 2 |  | 2 |  | 4 |  | 4 |  |

Abbreviations: ITE, institute of technical education; NITEC, national institute of technical education certificate; GCE, General Certificate of Education; SGD, Singapore dollar.

**Table S2. Details of structural and functional MRI protocols**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Sample size | TR (ms) | TE (ms) | TI (ms) | Flip angle (°) | Acquisition matrix | FOV (mm2) | Slices thickness (mm) | Number of slices | Time (mins) | Volumes |
| sMRI |  |  |  |  |  |  |  |  |  |  |  |
| Time 1 (4.5 years of age) | 87 | 2000 | 2.08 | 877 | 9 | 192 × 192 | 192 × 192 | 1.0 | 160 | 3.5 | - |
| Time 2 (6.0 years of age) | 136 | 2000 | 2.08 | 877 | 9 | 192 × 192 | 192 × 192 | 1.0 | 160 | 3.5 | - |
| Time 3 (7.5 years of age) | 203 | 2000 | 2.08 | 877 | 9 | 192 × 192 | 192 × 192 | 1.0 | 192 | 3.5 | - |
| Time 4 (10.5 years of age) | 216 | 2000 | 2.08 | 877 | 9 | 192 × 192 | 192 × 192 | 1.0 | 192 | 3.5 | - |
|  |  |  |  |  |  |  |  |  |  |  |  |
| rsfMRI |  |  |  |  |  |  |  |  |  |  |  |
| Time 1 (4.5 years of age) | 72 | 2660 | 27 | - | 90 | 64 × 64 | 192 × 192 | 3.0 | 48 | 5.32 | 120 |
| Time 2 (6.0 years of age) | 101 | 2660 | 27 | - | 90 | 64 × 64 | 192 × 192 | 3.0 | 48 | 5.32 | 120 |
| Time 3 (7.5 years of age) | 177 | 2620 | 27 | - | 90 | 64 × 64 | 192 × 192 | 3.0 | 48 | 5.24 | 120 |
| Time 4 (10.5 years of age) | 159 | 2620 | 27 | - | 90 | 64 × 64 | 192 × 192 | 3.0 | 48 | 5.24 | 120 |

Abbreviations: TR, repetition time; TE, time to echo; TI, inversion time; FOV, field of view.

**Table S3. Comparison of sample characteristics between the excluded participants and analytical sample (PSDQ-fMRI)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | Excluded (n = 51) | Included (n = 256) | P |
| Child sex, N (%) | Girl | 19 (37.3) | 136 (53.1) | 0.04 |
|  | Boy | 32 (62.8) | 120 (46.8) |  |
| Marital status, N (%) | Living with husband | 46 (93.9) | 246 (98.0) | 0.13 |
|  | Not living with husband | 3 (6.1) | 5 (2.0) |  |
|  | Missing | 2 | 5 |  |
| Maternal ethnicity, N (%) | Chinese | 27 (52.9) | 134 (52.3) | 0.24 |
|  | Malay | 12 (23.5) | 83 (32.4) |  |
|  | Indian | 12 (23.5) | 39 (15.2) |  |
| Household highest education, N (%) | Primary or secondary | 7 (15.9) | 30 (15.3) | 0.87 |
|  | ITE/MITEC | 5 (11.4) | 31 (15.8) |  |
|  | GCE A levels/ Polytechnic/ Diploma | 13 (29.6) | 60 (30.6) |  |
|  | University (Bachelor, Master, PhD) | 19 (43.2) | 75 (38.3) |  |
|  | Missing | 7 | 60 |  |
| Household monthly income, N (%) | SGD 0 - 1,999 | 7 (14.3) | 36 (15.1) | 0.14 |
|  | SGD 2,000 - 3,999 | 13 (26.5) | 83 (34.9) |  |
|  | SGD 4,000 - 5,999 | 20 (40.8) | 59 (24.8) |  |
|  | SGD ≥ 6,000 | 9 (18.4) | 60 (25.2) |  |
|  | Missing | 2 | 18 |  |
| Maternal psychological symptoms, mean (SD) |  | 8.1 (7.8) | 6.3 (7.8) | 0.05 |
|  | Missing | 0 | 4 |  |
| Harsh parenting, mean (SD)a |  | 0.4 (0.9) | 0.0 (1.0) | 0.01 |
|  | Missing | 19 | 82 |  |
| Internalizing problems, mean (SD)a |  | -0.2 (0.8) | -0.1 (0.9) | 0.71 |
|  | Missing | 19 | 82 |  |
| Externalizing problems, mean (SD)a |  | 0.1 (0.7) | -0.1 (0.9) | 0.03 |
|  | Missing | 19 | 82 |  |
| Mean head motion during rsfMRI, mean (SD) |  | 0.3 (0.1) | 0.1 (0.1) | <0.01 |
|  | Missing | 41 | 155 |  |

Abbreviations: ITE, institute of technical education; NITEC, national institute of technical education certificate; GCE, General Certificate of Education; SGD, Singapore dollar; sMRI, structural magnetic resonance imaging; rsfMRI, resting-state functional magnetic resonance imaging. a: these measures were standardized.

**Table S4. Associations between harsh parenting and child depressive symptoms**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Total (n = 254) | | |  | Girls (n = 113) | | | Boys (n = 141) | | | Pint |
|  | β | 95% CI | P-values |  | β | 95% CI | P-values | β | 95% CI | P-values |
| Emotional dimension | -0.02 | -0.12 to 0.09 | 0.79 |  | 0.00 | -0.16 to 0.17 | 0.96 | -0.08 | -0.23 to 0.07 | 0.31 | 0.66 |
| Functional dimension | -0.03 | -0.14 to 0.07 | 0.53 |  | 0.01 | -0.15 to 0.17 | 0.92 | -0.09 | -0.24 to 0.06 | 0.25 | 0.39 |

Model adjusted for child sex, child age at psychological outcome measurement, maternal ethnicity, household education, and maternal depressive symptoms (BDI) at 4.5YR.

**Table S5. Descriptive statistics of harsh parenting and child behavioral measures**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Internalizing behavior (4YR) | Internalizing behavior (10.5YR) | Externalizing behavior (4YR) | Externalizing behavior (10.5YR) | Harsh parenting (4.5YR) | Harsh parenting (10.5YR) |
| Girls | 10.19 (7.69) | 5.59 (5.63) | 11.90 (7.78) | 4.95 (5.00) | 2.25 (0.96) | 1.93 (0.85) |
| Boys | 9.30 (6.91) | 4.06 (3.77) | 11.92 (8.40) | 5.18 (5.05) | 2.33 (0.86) | 1.89 (0.72) |

The mean (SD) of the raw score is shown. Internalizing (4YR): 0-72, Internalizing (10.5YR): 0-64, Externalizing (4YR) 0-48, Externalizing (10.5YR): 0-70, Harsh parenting (4.5, 10.5YR): 0-5.

**Table S6. Associations between harsh parenting and child behavioral measures**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Girls | | 1 | 2 | 3 | 4 | 5 |
| 1 | Internalizing problems at 4 years |  |  |  |  |  |
| 2 | Internalizing problems at 10.5 years | 0.29 |  |  |  |  |
| 3 | Externalizing problems at 4 years | 0.69 | 0.18 |  |  |  |
| 4 | Externalizing problems at 10.5 years | 0.15 | 0.66 | 0.37 |  |  |
| 5 | Harsh parenting at 4.5 years | -0.12 | 0.04 | 0.00 | 0.23 |  |
| 6 | Harsh parenting at 10.5 years | -0.09 | -0.03 | 0.10 | 0.16 | 0.40 |
|  |  |  |  |  |  |  |
| Boys | | 1 | 2 | 3 | 4 | 5 |
| 1 | Internalizing problems at 4 years |  |  |  |  |  |
| 2 | Internalizing problems at 10.5 years | 0.37 |  |  |  |  |
| 3 | Externalizing problems at 4 years | 0.66 | 0.37 |  |  |  |
| 4 | Externalizing problems at 10.5 years | 0.28 | 0.51 | 0.44 |  |  |
| 5 | Harsh parenting at 4.5 years | 0.06 | 0.05 | 0.13 | 0.10 |  |
| 6 | Harsh parenting at 10.5 years | 0.14 | 0.05 | 0.26 | 0.17 | 0.44 |

Pearson correlation coefficients are shown.

**Table S7. Associations between harsh parenting and developmental trajectory of amygdala volume**

|  |  |  |  |
| --- | --- | --- | --- |
| (mm3) | B | 95%CI | P-values |
| **Total (n = 296)** |  |  |  |
| Harsh parenting | -6.64 | -25.35 to 12.08 | 0.49 |
| Age | 38.79 | 34.03 to 43.55 | <0.01 |
| Harsh parenting × age | 0.31 | -2.69 to 3.32 | 0.84 |
|  |  |  |  |
| **Girls (n = 151)** |  |  |  |
| Harsh parenting | -5.82 | -29.83 to 18.19 | 0.63 |
| Age | 44.14 | 38.27 to 50.01 | <0.01 |
| Harsh parenting × age | 0.91 | -2.90 to 4.72 | 0.64 |
| **Boys (n = 145)** |  |  |  |
| Harsh parenting | -5.77 | -35.70 to 24.15 | 0.71 |
| Age | 33.64 | 26.09 to 41.19 | <0.01 |
| Harsh parenting × age | -0.11 | -4.75 to 4.53 | 0.96 |

Model adjusted for child sex, maternal ethnicity, mean head motion, household education, and maternal depressive symptoms (BDI) at 4.5YR.

Random intercepts were included.

Results were for fixed effects aggregated across imputed datasets using Rubin's rule.

**Table S8. Associations between harsh parenting and developmental trajectories of global brain volumes**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Cerebral white matter (× 1000 mm3) | | |  | Cortical gray matter (× 1000 mm3) | | |  | Subcortical gray matter (× 1000 mm3) | | |
|  | B | 95%CI | P-values |  | B | 95%CI | P-values |  | B | 95%CI | P-values |
| **Total (n = 296)** |  |  |  |  |  |  |  |  |  |  |  |
| Harsh parenting | 2.03 | -4.85 to 8.92 | 0.56 |  | 2.15 | -7.71 to 12.01 | 0.67 |  | 0.18 | -0.22 to 0.58 | 0.37 |
| Age | 7.36 | 6.76 to 7.95 | <0.01 |  | -5.88 | -6.77 to -4.99 | <0.01 |  | 0.34 | 0.25 to 0.42 | <0.01 |
| Harsh parenting × age | -0.06 | -0.45 to 0.33 | 0.77 |  | 0.11 | -0.47 to 0.69 | 0.71 |  | -0.01 | -0.06 to 0.04 | 0.76 |
|  |  |  |  |  |  |  |  |  |  |  |  |
| **Girls (n = 151)** |  |  |  |  |  |  |  |  |  |  |  |
| Harsh parenting | 2.00 | -6.25 to 6.06 | 0.98 |  | 3.62 | -19.73 to 26.97 | 0.76 |  | 0.08 | -0.54 to 0.69 | 0.80 |
| Age | 7.06 | 6.18 to 7.95 | <0.01 |  | -6.12 | -7.37 to -4.88 | <0.01 |  | 0.32 | 0.22 to 0.42 | <0.01 |
| Harsh parenting × age | 0.11 | -0.49 to 0.71 | 0.72 |  | 0.25 | -0.58 to 1.09 | 0.55 |  | 0.01 | -0.05 to 0.08 | 0.74 |
| **Boys (n = 145)** |  |  |  |  |  |  |  |  |  |  |  |
| Harsh parenting | 2.13 | -28.22 to 32.48 | 0.89 |  | 0.96 | -31.04 to 32.96 | 0.95 |  | 0.23 | -0.39 to 0.85 | 0.47 |
| Age | 7.67 | 6.86 to 8.48 | <0.01 |  | -5.63 | -6.93 to -4.33 | <0.01 |  | 0.36 | 0.22 to 0.49 | <0.01 |
| Harsh parenting × age | -0.22 | -0.73 to 0.29 | 0.40 |  | -0.04 | -0.08 to 0.08 | 0.93 |  | -0.02 | -0.11 to 0.06 | 0.60 |

Model adjusted for child sex, maternal ethnicity, household education, and maternal depressive symptoms (BDI) at 4.5YR.

Model for subcortical gray matter and hippocampus volume additionally adjusted for estimated intracranial volume.

Random intercept is included.

Results are for fixed effects aggregated across imputed datasets using Rubin's rule.

**Table S9. Descriptive statistics of the changes in amygdala-based measures by age**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Amygdala volume | Amygdala-Hippocampus RSFC | Amygdala-ACC RSFC | Amygdala-NAcc RSFC | Amygdala-OFC RSFC | Amygdala-DLPFC RSFC | Amygdala-PPC RSFC |
| Girls, mean (SD) | 1.91e-15 (0.37) | -4.38e-15 (0.42) | 1.71e-15 (12.59) | 5.89e-15 (7.76) | -9.45e-15 (13.06) | 3.21e-15 (2.19) | 4.50e-15 (8.28) |
| Boys, mean (SD) | 2.20e-14 (0.55) | 4.96e-16 (0.53) | -4.23e-15 (11.52) | -4.31e-15 (6.75) | 2.21e-15 (12.29) | -1.05e-15 (2.17) | -8.72e-16 (8.98) |

**Table S10. Causal mediation analysis exploring the mediating role of amygdala-ACC RSFC slope in the associations between harsh parenting and externalizing problems in girls (n = 85)**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Harsh parenting → Amygdala-ACC RSFC slope → Externalizing problems | | |
|  | β | 95%CI | P-value |
| ACME | 0.06 | 0.01 to 0.14 | 0.02 |
| ADE | 0.25 | 0.004 to 0.48 | 0.04 |
| Total effect | 0.32 | 0.04 to 0.56 | 0.01 |
| % mediated | 0.20 | 0.05 to 0.83 | 0.02 |

ACME: average causal mediation effect, ADE: average direct effect.

Standard errors were estimated using a nonparametric bootstrap with 1,000 simulations.

Analyses were conducted using the imputed dataset obtained from the single imputation using the Expectation-Maximization algorithm.

Model adjusted for child age at outcome measurement, maternal ethnicity, household education, and maternal psychological symptoms.

**Reference**

Fan, F., Liao, X., Lei, T., Zhao, T., Xia, M., Men, W., Wang, Y., Hu, M., Liu, J., Qin, S., Tan, S., Gao, J.-H., Dong, Q., Tao, S., & He, Y. (2021). Development of the default-mode network during childhood and adolescence: A longitudinal resting-state fMRI study. *NeuroImage*, *226*(117581), 117581. https://doi.org/10.1016/j.neuroimage.2020.117581

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