Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. Detailed Methodology

Participants
A total 204 mother-infant dyads participated in the study. MRI scans we collected for 103 infants, five of which were excluded due to apparent imaging artifacts due to excessive head motion. The final study sample thus consisted of 98 mother-infant dyads. A total of 101 infants were not scanned due to: 10 declined MRI session, 10 withdrew, 13 scanner equipment problem, 8 lost to follow up, 14 dropped due to medical complications, 18 scheduling logistics issues, 30 investigators' decision not to scan as a robust number of infants already in N–PMD group relative to expense of scanning. There were no significant differences in demographic variables between the dyads (n=103) with and without (n= 101) infant MRI scans (see **eTable 1**)

SSRI dosage and duration
The majority (n=13) of mothers in the SSRI group reported taking the medication throughout all three trimesters of their pregnancy. There was, however, variance in the dosages prescribed (**Table 1**)**.** To analyze dosage effects, mothers were binned into low (Fluoxetine≤10mg; Escitalopram≤5mg; Citalopram≤10mg; Sertraline≤50mg; Venlafaxine≤75mg) mid (Fluoxetine11-40mg; Escitalopram 6-15mg; Citalopram 11-30mg; Sertraline 51-150mg; Venlafaxine 76-125mg) and high (Fluoxetine>40mg; Escitalopram >15mg; Citalopram >30mg; Sertraline>150mg; Venlafaxine>125mg) SSRI dosage categories.

5-HTTLPR genotyping
The region encompassing 5-HTTLPR and rs25531 polymorphisms was amplified with primers; FORWARD: 5′TCCTCCGCTTTGGCGCCTCTTCC-3′; REVERSE: 5′-TGGGGGTTGCAGGGGAGATCCTG-3′via a polymerase chain reaction in multiplex master mix (Qiagen, Calif., USA). Amplicon was resolved on a 2.3% UltraPure™ Agarose (Invitrogen, Carlsbad, CA), and visualized under the UV trans-illuminator. Here, 512 bp and 469 bp bands were called as L and S allele at 5-HTTLPR respectively.

MRI Acquisition
For dMRI, with a minimum possible run duration, both voxel-wise in-plane and angular (i.e., number of non-collinear directions) resolutions were considered. Given the small size of infant brains and under-development of the white matter fascicules, parameters were selected to optimize voxel-wise, in-plane resolution (i.e., sub-millimeter), while maintaining sufficient angular resolution. Considering this, our imaging parameters were: for T2-weighted structural images, PROPELLER (Periodically Rotated Overlapping Parallel Lines with Enhanced Reconstruction) sequence, voxel size $0.74 \times 0.74 \times 1$ mm^3, dimensions 256 x 256 x 108, FOV 190mm, TR/TE 11832/100 ms, flip angle 142 degrees; and for diffusion MRI: voxel size $0.74 \times 0.74 \times 2$ mm^3; dimensions 256 x 256 x 56; FOV 190 mm; slices 50, TR/TE 8000/83.4ms, flip angle 90 degrees, and 3 images without diffusion weighting (b0) and 11 images with diffusion weighting along non-collinear directions (b=600 $s\,m^{-2}$). During scanning, and after each run, images were visually inspected for severe motion-related artifacts.

In the case of an infant waking up, the MRI session was paused until he/she fell asleep again; scanning then resumed. A typical MRI session took about two hours. To ensure acquisition of high-quality data with limited head motion, propeller T2 scanning was repeated up to twice and dMRI, up to six times. For each infant, the subsequent MRI analyses were conducted on the single run from each modality with the least amount of head motion.

Volume-by-Volume Quality Assessment in DWI
A trained researcher (VB) examined every volume of DWI in each subject for severe artifact that were persistent to our preprocessing pipeline. 27 infants had at least one bad volume to be excluded. No group differences were found in the number of unusable volumes (**Table S1**).

Structural MRI Analysis: Voxel-Based Morphometry
We found that a typical single VBM pipeline, either in SPM or FSL, failed to produce quality brain extraction and segmentation due to issues specific to infant structural scans (e.g., a thin skull or lack of contrast). Therefore, we systematically tested different analytic methods (e.g., several widely-used methods of brain extraction or tissue segmentation in FSL or SPM) and chose the combination that generated the best quality GM estimation. For this, in each analysis step, trained researchers (CL-C and VB), who were blind to group assignment, visually inspected the

quality of every output. The following methods were adopted to ensure accuracy of VBM in terms of segmentation and template estimation (Figure S1). First, T2-weighted images underwent Non-parametric Non-uniform intensity Normalization (N3) correction (RRID:SCR_002484)[1] For brain extraction, N3-corrected images underwent Pediatric Brain Extraction Using Learning-based Meta-algorithm[2]. As we assessed individual output, this algorithm turned out to be superior to other brain extraction tools available in FSL or BrainSuite (http://brainsuite.org/processing/surfaceextraction/bse/). Nevertheless, it failed in several subjects. In those subjects whose brain extraction was poor, we chose intermediate outputs of the meta-algorithm were superior to the final outputs.

Second, for tissue segmentation, we used the segmentation function in SPM 12 using tissue probability maps for the infant brain.[3] This method produced quality tissue segmentation, superior to "FMRIB's Automated Segmentation Tool" or FAST (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FAST). Two images were excluded as we failed to obtain acceptable GM segmentation due to image artifacts.

Third, for template generation and spatial normalization, segmented GM images underwent the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) toolbox[4] in SPM12 (Figure S1). This procedure involves estimation of deformations that best align individual GM images by iteratively registering them with their average (template generation). Estimated deformations are then used to spatially normalize (warp) Jacobian-scaled GM images.

Finally, spatially normalized and Jacobian scaled GM images were smoothed using 4mm Gaussian Full-Width-Half-Maximum (FWHM) kernel. We reasoned that, given the smaller infant brain size, a smaller smoothing kernel should be used (e.g., smaller than the 8mm FWHM that is typically used in adult brain analysis). Nevertheless, to avoid any smoothness-specific effects, we confirmed the results with 8mm FWHM.

Diffusion MRI Analysis: Probabilistic Tractography

Before preprocessing, a trained researcher (VB), blind to group assignment, manually checked every volume in each subject for motion-related artifacts that were difficult to detect algorithmically in the infant brain images. These volumes were excluded in the subsequent preprocessing and analysis. In 27 infants, we found at least one unusable volume with subject-specific artifacts; this count did not differ by group ($P > 0.9$; $\chi^2 = 0.728$).

DWI was preprocessed using the following pipeline in MRtrix. DWI was first denoised using a novel algorithm based on Random matrix theory that permits data-driven, non-arbitrary threshold for Principal Component Analysis denoising; this method enhances the DWI quality for quantitative and statistical interpretation [5]. Denoised images then underwent eddy current and motion correction,[6] brain extraction from three non-diffusion-weighted images (taking their median), and bias field correction using N4 algorithm (N4ITK), an improved N3 method, in ANTS.[7] We then estimated fiber orientation distributions from each preprocessed data using 2nd-order integration over fiber orientation distributions (iFOD2). Based on the FODs, probabilistic tractography was performed using constrained spherical devolution (CSD). We used a target streamline count of 10 million across the whole brain. The tractograms were filtered using SIFT with a target streamline count of 3 million. After a primary statistical analysis using these filtered tractograms, we tested whether effects of interest were robust to the tractography and filtering parameters, such as the target streamline count for tractography, SIFT, or a ratio between them. This method permits mapping streamline estimation back to individual's DWI, and updating a reconstruction to improve model fit. This approach renders the streamline counts connecting two brain regions proportional to the total cross-sectional area of the white matter fibers connecting those regions, enhancing streamline counts as a biologically plausible quantity, representing 'structural connectivity'. This was done by repeating tractography and SIFT with a set of extreme parameters (100 million and 5 million target streamlines, respectively) with a filtering factor of 20 (100/5). This generated tractograms of about 100GB per subject (cumulatively more than 9TB for the entire group) with average CPU time of 144 hours (13,000 hours across subjects).

We generated a connectivity matrix from 90 brain regions in each infant from the final filtered tractograms. We used streamline counts as the primary connectivity metric in this study as in a recent human infant imaging study [8], as well as fractional anisotropy (FA) and mean diffusivity (MD) as secondary measures. A prior macaque study suggests the validity of streamline counts as an indicator of fiber connection strength, with the number of streamlines significantly correlating with tract-tracing strength in the macaque brain [9]. All the analysis codes will be available at online repository (https://github.com/jcha9928/neonatal_ssri_exposure)

Diffusion MRI Analysis: Generation of Anatomical Mask
Since a significant portion of infants (n=13) had no usable T2-weighted structural images, we decided not to perform individual brain segmentation and parcellation using the structural images. Instead, we opted to use a population-based neonatal brain atlas[3] to define anatomical ROIs for tractography. A standard infant structural image was warped into the individual diffusion space using non-linear Symmetric Normalization (SyN) algorithm in Advanced Normalization ToolS (ANTS; RRID: 004757), of which superior accuracy has been tested compared with a wide range of non-linear registration algorithms[10] (the same parameters for SyN was used in this study). We also confirmed its greater accuracy compared with FMRIB's Linear Image Registration Tool in FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FNIRT).

Diffusion MRI Analysis: Tract Based Spatial Statistics
Voxel-wise analysis on the white matter FA maps was performed using Tract-Based Spatial Statistics (TBSS) in FSL. Nonlinear registration (using fnirt program in FSL) was used to select the best target FA map that requires the minimum extent of warping among all FA maps. Images were nonlinearly registered to the selected target image and resampled to 1mm isometric voxels. Images were visually inspected for suboptimal registration. Resampled images were then used to extract a two-dimensional common white matter structure (so-called "skeleton") using tbss_skeleton program. The same general linear model as above was fitted to the final skeletonised FA maps using non-parametric randomisation (conditional Monte Carlo permutation test; 10,000) in FSL. Statistical results were corrected using the threshold-free cluster enhancement method at whole-brain level alpha < 0.05."

Statistical Analysis: Receiver Operating Characteristic (ROC) Analysis with Cross Validation
ROC (receiver operating characteristic) analysis with cross validation was performed to test the generalizability of the findings and to estimate the sensitivity of the selected brain measures in predicting prenatal exposure to SSRI. For VBM, the right amygdala and the right insula GM volume estimates, separately extracted from the VBM analysis (applying the anatomical atlas), were used; for structural connectivity, streamline counts between the right amygdala and the right insula were used. We estimated logistic regression with combinations of those brain measures as the predictors and with group as the response variable using leave-one-out cross-validation (LOOCV). Sensitivity (true positive rate) and specificity (true negative rate) of the models was calculated. Model performance was compared using Wilcoxon rank-sum test.

Statistical Analysis: Confounding Variables: The potential effect of sociodemographic variables was assessed using linear regression. Significance was determined via exhaustive permutation tests; each variable (socioeconomic status, maternal age, comorbid psychiatric conditions years of education, ethnicity, infant 5-HTTLPR genotype) was tested using a separate model in addition to the set of covariates previously described.

eResults. Further Results

Potential Confounds of Socioeconomic Status

Given the group differences in SES, of which associations with brain development have been documented[11], we sub-sampled our participants to match on SES by excluding non-SSRI participants with lower SES (after sub-sampling, mean SES in SSRI=3·5 vs. mean SES in non-SSRI=3·4). In the SES-matched sub-samples (44 infants for structural MRI and 45 for diffusion MRI), the effects of prenatal exposure to SSRI remained significant (amygdala GM volume: $t = 2{\cdot}739$, $p = 0{\cdot}009$; insula GM volume: $t = 3{\cdot}214$, $p = 0{\cdot}003$; amygdala-insula WM connectivity: $t = 3{\cdot}727$, $p = 0{\cdot}0008$; linear regression and permutation to determine significance).

Dosage Effects

We found non-significant dosage effects on the selected brain measures in the SSRI group (n=13): amygdala GM volume ($t = 0.31$; $p > 0.77$; partial eta square = 0.015), insula GM volume ($t = 0.96$; $p > 0.37$; partial eta square = 0.133), amygdala-insula structural connectivity ($t = -0.5$; $p > 0.6$; partial eta square = 0.039) (permutation testing with linear models; the same covariates were included). Though none reached statistical significance, a positive association between an increase in insula GM volume and a higher dosage showed a medium effect size. Due to the lack of variability in the duration of SSRI exposure, we were unable to examine the effects of timing or duration of exposure.

Tract Based Spatial Statistics

TBSS pipeline generated acceptable spatial normalization except for two scans, which were excluded from the statistical analysis. Randomization permutation showed no significant effects of SSRI on white matter FA tracts at $p < 0.05$ (corrected at the whole brain level)."

eReferences

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eTable 1. Comparison of Demographic Variables Between Subjects With and Without Infant MRI Scans (Related to Participants).

Name	Subjects without MRI (n = 101)	Subjects with MRI (n = 103)	Test Statistic (df)	*p*
Gestational age at birth, mean (SD), weeks	38.53 (5.80)	39.31 (1.08)	F (1,197) = 1.80	0.18
Sex			X^2(2) = 0.16	0.67
Male	42	48		
Female	54	55		
Infant Birth Weight, mean (SD), grams	3294.11 (775.21)	3212.36 (531.26)	F (1,191) = 0.74	0.39
Maternal Age	31.22 (5.75)	30.57 (5.99)	F (1,202) = 0.62	0.43
Maternal Race/Ethnicity			X^2(3) = 5.31	0.15
Hispanic/Latina	16	28		
White/Caucasian	44	32		
Black/African American	26	28		
Other	13	15		
Total Family Income			X^2(3) = 4.81	0.19
$0–25,000	29	28		
$26,000–50,000	12	24		
$51,000–100,000	31	24		
$100,001+	25	26		
Maternal CES–D (prenatal), mean (SD)	12.87 (10.79)	12.08 (9.55)	F (1,202) = 0.30	0.58
Maternal CES–D (postnatal), mean (SD)	10.05 (9.24)	9.57 (8.02)	F (1,145) = 0.10	0.76

eTable 2. Number of Infants Having Unusable Volumes of DWI (Related to Results-Diffusion MRI Analysis-Probabilistic Tractography).

Group	# of participants having 1 unusable volume	# of participants having 2 unusable volumes	# of participants having 3 unusable volumes
SSRI	2	1	0
PMD	4	3	0
HC	9	7	1

HC, health controls; PMD, prenatal maternal depression; SSRI, selective serotonin reuptake inhibitors

eTable 3. Parameter Estimates Adjusting for Confounding Variables. (Related to Results-Potential Confounds)

Brain measures	Covariate	Effects of Covariates on Brain Phenotypes		Adjusted Effects of Group on Brain Phenotypes	
		b (95% CI)	significance*	b (95% CI)	significance
Right Amygdala GM	Socioeconomic status	3e-03 (-5e-03 ~ 1e-02)	*t*=0.82; *P*=0.41	1e-02 (3e-03 ~ 2e-02)	*t*=2.64; *P*=0.009**
	Years of Education	1e-03 (-4e-03 ~ 7e-03)	*t*=0.42; *P*=0.67	2e-02 (5e-03 ~ 3e-02)	*t*=2.83; *P*=0.006**
	Ethnicity†	n.a.	*F*=0.93; P=0.43	2e-02 (7e-03 ~ 2e-01)	*t*=3.04; *P*<0.001***
	Comorbidity	-2e-03 (-8e-03 ~ 3e-03)	*t*=0.85; *P*=0.40	2e-02 (5e-03 ~ 3e-02)	*t*=2.91; *P*=0.005**
	infant 5-HTTLPR SNP	3e-05 (-7e-03 ~ 8e-03)	*t*=0.008; *P*=0.99	2e-02 (4e-03 ~ 3e-02)	*t*=2.74; *P*=0.008**
Right Insula GM	Socioeconomic status	6e-05 (-8e-04 ~ 9e-04)	*t*=0.13; *P*=0.89	1e-03 (6e-04 ~ 3e-03)	*t*=3.25; *P*=0.002**
	Years of Education	-2e-04 (-7e-04 ~ 3e-04)	*t*=0.79; *P*=0.43	2e-03 (9e-04 ~ 3e-03)	*t*=3.70; *P*<0.001***
	Ethnicity	n.a.	*F*=1.11; P=0.35	2e-03 (9e-04 ~3e-03)	*t*=3.80; *P*=0.003*
	Comorbidity	7e-05 (-5e-04 ~ 6e-04)	*t*=0.25; *P*=0.79	2e-03 (6e-04 ~ 3e-03)	*t*=3.21; *P*=0.002**
	infant 5-HTTLPR	-1e-04 (-8e-04 ~ 6e-04)	*t*=-0.30; *P*=0.76	2e-03 (5e-04 ~ 3e-03)	*t*=3.05; *P*=0.003**
Right Amygdala-Right Insula WM connectivity	Socioeconomic status	-3.5 (-32.8 ~ 25)	*t*=-0.25; *P*=0.80	65.3 (113.0 ~ 171.2)	*t*=3.57; *P*<0.001***
	Years of Education	-2.1 (-19.3 ~ 1.5)	*t*=-0.23; *P*=0.81	65.2 (30.3 ~ 100.1)	*t*=3.71; *P*<0.001***
	Ethnicity	n.a.	*F*=1.49; P=0.23	58.6 (114.3 ~ 172.1)	*t*=3.38; *P*=0.001**
	Comorbidity	-1.1 (-18.2 ~ 16.1)	*t*=-0.25; *P*=0.80	69.8 (35.7 ~ 103.9)	*t*=4.10; *P*<0.001***
	infant 5-HTTLPR	11.2 (-12.9 ~ 35.4)	*t*=0.92; *P*=0.35	80.6 (47.3 ~ 113.9)	*t*=4.81; *P*<0.001***

*t test against to zero; † ethnicity is coded as a factor variable and its significance was tested in ANOVA (*F* statistics) comparing the models with and without it.

