Supporting Information

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SI Methods

Participants. Recruitment for the Wisconsin Study of Families and Work began in 1990 and was designed to gather information on parental leave and health outcomes from a subsample of the general population in and around two cities in southern Wisconsin where the woman was working either outside the home or as a full-time homemaker. A total of 570 women and their partners initially were recruited from clinics and hospitals while attending routine prenatal visits. Mothers had to be over 18 y old, in their second trimester of pregnancy, and living with the baby's biological father. Selection for the present study was based on proximity to the laboratory and MRI exclusionary criteria. Participants' (n = 64) racial background was 61 white, 1 Native American/Alaskan, and 2 African American. Resting-state data were collected during a 4-h laboratory visit. Informed consent (and parental permission in childhood) was obtained for all assessments, and participants received monetary compensation for participating. University of Wisconsin-Madison Institutional Review Boards approved all procedures.

Imaging Data Acquisition and Processing. Structural and functional images were collected on a 3T MRI scanner (Discovery MR750, General Electric Medical Systems) with an eight-channel RF head coil array. T1-weighted structural images (1-mm^3 voxels) were acquired axially with an isotropic MPRAGE sequence (TE = 3.18 ms, TR = 8.13 ms, TI = 450 ms, flip angle = 12°). Subjects were instructed to rest silently with their eyes closed while remaining "clear, calm, and awake" during the collection of a T2*-weighted gradient-echo echo-planar pulse sequence lasting 420 s (210 volumes) with a TE, TR, and flip angle of 25 ms, 2,000 ms, and 60°, respectively. Image volumes had a resolution of 3.5 × 3.5 × 5 mm^3 (matrix size = 64 × 64; 30 sagittal slices).

Most data-reduction steps were performed using the Analysis of Functional and Neural Images (AFNI) software package (1). Images were corrected for slice-dependent time shifts and motion (2) and were field-map corrected using FMRIB Software Library (FSL) PRELUDE (3, 4) and in-house software. Anatomical images then were aligned to the fifth volume of echo-planar image (EPI) time series using a Local Pearson Correlation cost function (5). The first four volumes of the time series were removed because of T1-equilibrium effects. Both anatomical and functional images were transformed to Talairach Atlas space using a nine-parameter affine transformation and were resampled to 2 × 2 × 2 mm voxels.

Resting-state fMRI time courses were temporally filtered (bandpass: 0.001 Hz < f < 0.01 Hz). In a step to reduce the influence of motion further, time points were censored if the motion of a point 87 mm from the center of rotation was greater than 2 mm per degree. Note that the Childhood Trauma Questionnaire (CTQ) was not associated with subject motion or with the number of time points censored (see below and Table S3). Variance from sources of noninterest was removed using multiple linear regression (AFNI's 3dDeconvolve function). Six rigid body-motion parameters were included as nuisance regressors, along with the average signal (calculated from all voxels) and derivatives from both eroded cerebral spinal fluid (CSF) and 2× eroded white matter (WM) masks. Masks were generated with an automated segmentation of the T1-weighted structural scan using FSL's FAST routine (3, 4, 6) and were transformed to Talairach Atlas space. EPI time series then were spatially smoothed with a 6-mm FWHM Gaussian kernel (postnuisance regression to avoid partial volume averaging within CSF and WM masks).

Functional Connectivity Analyses. Resting-state functional connectivity (rs-FC) estimates were computed using a seed region-based approach (7). Binary masks of the left and right amygdala and hippocampus were defined in AFNI by placing spheres with a 4-mm radius at the locations of the amygdala and hippocampus according to the Talairach Daemon (8). The average preprocessed functional MRI (fMRI) signal-intensity time course over each amygdala region of interest then was regressed against the signal-intensity time courses of all other voxels in the brain. Time points were motion censored as outlined above. The correlation coefficient of each voxel in the resultant statistical parametric maps was converted into a z-score using the Fisher z-transformation. Participant connectivity maps then were entered into two-tailed regressions (AFNI's 3dttest++) while covarying CTQ scores, childhood basal cortisol levels, and/or other behavioral variables of interest. Cluster sizes were selected based on α significance values ≤0.05 and were estimated with AFNI's 3dClustStim and 3dFWHMx. In our data, at an individual voxel $P < 0.001$, a minimum cluster size of 111 voxels is required to have a corrected $P \leq 0.05$. Before any behavioral covariates were included, both the left and right amygdala showed significant positive rs-FC with the contralateral amygdala, dorsomedial prefrontal cortex and ventrolateral prefrontal cortex (PFC), ventral striatum, superior temporal gyrus, and ventromedial prefrontal cortex (vmPFC). Both left and right hippocampus showed significant positive rs-FC with the contralateral hippocampus, ventromedial and dorsomedial PFC, ventral striatum, posterior cingulate, bilateral angular gyrus, middle and superior temporal gyrus, and postcentral gyrus (PCG).

Path Modeling. Mplus software (version 5.2) (9) was used to construct a structural equation model (SEM) testing the mediating effects of brain connectivity in the association between childhood experiences of maltreatment and persistent internalizing symptoms. SEM provides a variety of measures indicating the associations between variables and the overall fit of the proposed model. Models must consider the number of participants relative to the number of paths being estimated; a 5:1 ratio is considered acceptable (10). A nonsignificant χ^2 test is desirable, because it indicates that the proposed model is not statistically significantly different from the observed data (11). Fit indices such as the root mean square error of approximation and the standardized root mean square residual generally are considered adequate below 0.08–0.10 (12). The comparative fit index is sensitive to the number of estimated paths in the model and is considered good at 0.93 or above.

Association of Adolescent Report of Childhood Experiences of Maltreatment with Earlier Maternal Reports of Family Stress. The retrospective nature of the CTQ can make it subject to memory bias. Thus, we took advantage of the longitudinal study design to investigate associations of the adolescent-reported CTQ score with earlier maternal reports of family stress obtained during the childhood and early adolescent years. A stepwise regression analysis performed within SPSS (v. 21) revealed that the CTQ score was substantially predicted by maternal reports of financial stress during infancy/preschool, negative parenting in late childhood, and financial stress during adolescence (model fit R^2 = 0.32, $P < 0.001$; Table S1). In addition, the effect of financial stress during infancy/preschool dropped to nonsignificance after the inclusion of financial stress during adolescence, suggesting mediation. These results demonstrate that adolescent reports of

experiences of maltreatment during childhood substantially reflect earlier maternal reports of family stress.

Childhood Experiences of Maltreatment and Adolescent Amygdala rs-FC with Brain Regions Not Predictive of Adolescent Internalizing Symptoms. As shown in Table 1, the CTQ score also significantly predicted connectivity between amygdala and additional brain regions that did not predict adolescent internalizing symptoms (see below). The CTQ score predicted lower connectivity between left and right amygdala and right PCG ($P < 0.01$, $R^2 = 0.32$ and 0.28, respectively) and greater connectivity between left amygdala and left dorsolateral prefrontal cortex (dlPFC) ($P < 0.02$, $R^2 = 0.35$). ANCOVA of the extracted rs-FC revealed main effects of the CTQ score only for left amygdala–PCG ($F_{1,60} = 21.84$, $P < 0.001$) and left amygdala–dlPFC rs-FC ($F_{1,60} = 23.79$, $P < 0.001$). Right amygdala–PCG rs-FC showed a significant sex by CTQ score interaction ($F_{1,60} = 4.55$, $P = 0.037$). This effect was driven by a stronger association with CTQ score in females ($R^2 = 0.61$ in females and $R^2 = 0.03$ for males). These findings remained significant when controlling for adolescent current life stress (left amygdala–PCG CTQ score main effect: $F_{1,59} = 20.07$, $P < 0.001$; left amygdala–dlPFC CTQ score main effect: $F_{1,59} = 20.41$, $P < 0.001$; right amygdala–PCG sex–CTQ score interaction: $F_{1,59} = 4.33$, $P = 0.04$).

Correlations Between Childhood Experiences of Maltreatment, Adolescent Internalizing Symptoms, and Functional Connectivity. Table S2 shows the bivariate correlations between childhood experiences of maltreatment, adolescent internalizing symptoms, and extracted functional connectivity values derived from regions associated with CTQ score. Analyses were controlled for sex. Amygdala– subgenual anterior cingulate cortex (sgACC) and hippocampus–sgACC connectivity were significantly correlated with adolescent internalizing symptoms. Amygdala–PCG and amygdala–dlPFC functional connectivity were not significantly correlated with adolescent internalizing symptoms. There was a trend for an association between left amygdala–PCG connectivity and internalizing symptoms ($P = 0.06$). To examine whether this pathway contributes to internalizing symptoms independently of amygdala/hippocampus–sgACC connectivity, we performed an additional partial correlation analysis with sex and total amygdala/hippocampus–sgACC connectivity included as variables. When controlled for left amygdala–PCG connectivity, the association between total amygdala/hippocampus–sgACC connectivity and internalizing dropped only modestly (from −0.31 to −0.23; $P = 0.07$). However, when controlled for total amygdala/hippocampus–sgACC connectivity, the association between left amygdala–PCG connectivity and internalizing was no longer present (from −0.24 to −0.11, $P = 0.38$). These analyses indicate that only amygdala–sgACC and hippocampus–sgACC connectivity remained as potential mediators between experiences of maltreatment during childhood and adolescent internalizing symptoms and therefore became the focus of the path modeling.

Subject Motion During Resting-State fMRI and Association with CTQ Total Score. Summary measures of motion for each subject were obtained by (*i*) computing the sum of the squared differences (SSD) (13) between successive time points of the six estimated motion parameters (three rotation, three translation) and (*ii*) computing the total number of time points censored. In this sample, there was very little subject motion (SSD = 0.097 ± 0.073 mm per time point; number of time points censored = 1 ± 5 of 210 total time points). Of the 64 subjects, 59 had no time points censored. The remaining 5 subjects had the following number of time points censored: 3, 3, 6, 11, and 41. Across subjects, there was no significant association between CTQ score and subject motion as assessed with either of these motion summary measures (Pearson's correlation between CTQ score and SSD = 0.021 and between CTQ score and number of time points censored = 0.031). These results are summarized in Table S3.

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Table S1. Association between adolescent reports of experiences of maltreatment during childhood (CTQ scores) with earlier maternal reports of family stress during childhood

Step	R^2	Variable	β	t	P
1	0.12	Infancy/preschool financial stress	0.35	2.90	0.005
2	0.23	Infancy/preschool financial stress	0.24	2.02	0.047
		Late childhood negative parenting	0.34	2.87	0.006
3	0.33	Infancy/preschool financial stress	0.00	0.00	0.997
		Late childhood negative parenting	0.26	2.26	0.027
		Early/midadolescence financial stress	0.42	2.92	0.005

A stepwise linear regression examined predictors of CTQ score by average maternal stress reports from three developmental periods: infancy/preschool, late childhood (age 9–11 y), and early/midadolescence (age 13–15 y). Maternal stress measures included maternal depression, negative parenting, marital conflict, maternal role overload, financial stress, and family anger.

Table S2. Bivariate correlations, controlled for sex, for extracted rs-FC estimates from regions in Table 1 with experiences of maltreatment during childhood (CTQ total score), and adolescent internalizing symptoms

		Experiences of maltreatment during childhood		Adolescent internalizing symptoms	
Seed	Identified cluster	r	P	r	P
Left hippocampus	sgACC (BA 25)	−0.63	<0.001	−0.29	0.02
Left amygdala	R. PCG (BA 4)	−0.56	<0.001	−0.24	0.06
	L. dlPFC (BA 10)	0.59	<0.001	0.13	0.32
Right amygdala	sgACC (BA 25)	−0.53	<0.001	−0.26	0.04
	R. PCG (BA 4)	−0.52	<0.001	−0.08	0.51

Table S3. Summary of subject motion parameters during resting state fMRI and association with CTQ total score

Metric	Mean value	Correlation with CTQ score, Pearson's r
SSD of motion parameters	0.097 ± 0.073 mm per time point	0.021
Number of time points censored (out of 210 time points)	1 ± 5	0.031

Summary measures of motion for each subject were obtained by computing the sum of the SSD between successive time points of the six estimated motion parameters and computing the total number of time points censored. There was very little subject motion, and neither measure was associated with CTQ total score.