Supporting Information

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SI Materials and Methods

Preliminary Analysis of the Subsample Used. To ensure that the subsample of participants who completed the MRI assessment was representative of the entire Bucharest Early Intervention Project (BEIP) sample, children who participated in the MRI assessment were compared with the children who did not participate within each of the three groups: care as usual group (CAUG), foster care group (FCG), never-institutionalized group (NIG). These groups were compared using a two-sample t test for differences in: age at this follow up visit, sex, full-scale intelligence quotient (IQ) at 8 y of age, internalizing and externalizing symptoms at 8 y, and α-power. IQ was measured using the Wechsler intelligence scale for children (WISC-IV), internalizing symptoms were measured via caregiver report of each child's symptoms of anxiety or depressive disorders using a structured clinical interview for young children, the Preschool Age Psychiatric Assessment (1). Externalizing symptoms including symptoms of attention-deficit/hyperactivity disorder, oppositional defiant disorder, and conduct disorder were assessed using the same interview. No group differences were found for any of these variables, with the exception that children in the NIG who were included in the MRI study had significantly more externalizing symptoms than those who were not included in the MRI study, $t(42) = 2.19$, $P = 0.03$, (MRI mean = 3.6 symptoms, SD = 3.33, no MRI mean = 1.2 symptoms, SD = 3.48). It may be that parents of children who were never institutionalized were slightly more likely to participate in this follow-up visit if they had some concerns about their child's well-being. (See Table S1 for a description of the sample). Overall, this analysis indicates that the participants in the MRI study are a representative subsample of the total sample of participants in the BEIP.

Details on Randomization. Initial randomization in the BEIP occurred for the 136 children residing in institutions at baseline and was performed by assigning each child a number from 1 to 136. Numbers were written on slips of paper and placed into a hat. The first number drawn from the hat was assigned to remain in the institution, the next was assigned to foster care, and so on, until all children had been assigned. Study design and methods of the BEIP have been described extensively elsewhere (2, 3).

Details on Foster Care Intervention. Because of a historical reliance on institutional care for abandoned children in Romania, foster care was almost nonexistent in Bucharest at the time the study began. As a result, we recruited and trained foster parents, using manuals developed by and for Romanians, to develop a network of 56 foster homes to care for 68 children randomized to foster care. BEIP foster parents received assistance from three project social workers who received initial training and weekly consultation from experienced mental health practitioners in the United States. The overall goal of the foster care intervention was to have foster parents make full psychological commitments to the children in their care. Through frequent and regular contacts, BEIP social workers carefully monitored the match between children and parents, encouraged child/parent attachment, made suggestions about managing common behavioral problems, and helped parents understand the special needs of formerly institutionalized children. Parents encouraged foster parents to develop a loving, committed relationship with their foster children. In fact, several foster parents decided to adopt the children they were fostering, even though this meant giving up a salary and becoming responsible for all future costs of the child. Stability of placements was 87% from initial placement (children were 7–33 mo of age) through age 54 mo. At that point, the intervention ended and financial and administrative support of foster parents was assumed by the local government authorities in Bucharest.

Details on FreeSurfer Analysis. FreeSurfer processing includes motion correction of a volumetric T1-weighted image, removal of nonbrain tissue using a hybrid watershed/surface deformation procedure (4), automated Talairach transformation, previously validated in pediatric populations (5), and segmentation of the subcortical white matter and deep gray matter volumetric structures, separately validated for use with pediatric populations (6).

FreeSurfer provided 15 volume measurements. We collapsed across several of these measurements to reduce multiple comparisons, measurement error, and because we did not have specific hypotheses at the level of detail automatically provided by the FreeSurfer program. In total, we examined nine structural volumes, including: total brain volume, total cortical gray matter, total white matter, thalamus, hippocampus, amygdala, basal ganglia (caudate, putamen, nucleus accumbens, and pallidum), posterior corpus callosum (CC) (posterior and midposterior CC), middle CC, and anterior CC (anterior and midanterior CC). See Fig. S1 for volume in the CC by group. See Table S2 for volumes of each structure by group membership.

Details on EEG Analysis. Before applying the EEG cap, the scalp underlying each electrode site was gently abraded and electrolytic conducing gel was inserted between the scalp and electrode. Impedances were measured at each site and were kept below 10 k ohms. Channels were digitized at 512 Hz using a 12-bit A/D converter (+2.5 V range) and Snap-Master acquisition software (HEM Data).

One channel of vertical electrooculogram (EOG) was recorded from electrodes placed above and below the left eye to record blinks and eye movements. The EEG and EOG signals were amplified by factors of 5,000 and 2,500, respectively, using custom bioelectric amplifiers from SA Instrumentation. Amplifier filter settings for all channels were 0.1 Hz (high pass) and 100 Hz (low pass). Before the recording of EEG from each participant, a 50-μV 10-Hz signal was input into each of the channels to and the amplified signal was recorded for calibration purposes.

Details on the Mediation Analysis. To test the significance of the mediation models, a nonparametric bootstrapping approach was used estimate the distribution of the change in the association of each predictor with the outcome after controlling for two mediators: total cortical gray matter and total white matter volume (5,000 bootstrap resamples) as described in Preacher and Hayes (7, 8; see also ref. 9). Generating this distribution allows the construction of a confidence interval from which the statistical significance of the indirect effect can be evaluated for each mediator separately. Confidence intervals that do not include zero indicate a significant indirect effect of the predictor on the outcome through the mediators. This approach has been recommended for use with small samples because it does not rely on assumptions of normality, which are often violated in small samples (8). Given the small sample size in this study for a mediation analysis, significance of mediation outcomes was assessed at a 90% confidence interval, making criteria for significance slightly more lenient.

Alternative Data Analysis: Amygdala. Studies have observed increased amygdala volume for children previously exposed to institutionalization or other forms of adversity during childhood. These studies have often used a different metric of amygdala volume than the one we use here: relative amygdala volume, defined as amygdala volume/total brain volume. To ensure that our findings are not limited by our methods, we also estimated the effect of institutionalization on relative amygdala volume. Relative amygdala volume was not significantly different for children in the EIG compared with children in the NIG ($B = 0.00064$, $t = 1.182$, $P = 0.24$); nor was it significantly different for children in the CAUG ($B = 0.00056$, $t = 0.961$ $P = 0.36$) or the FCG ($B = 0.00061$, $t = 0.959$, $P = 0.34$) compared with the NIG (Fig. S2). These findings were consistent weather or not we controlled for age and sex.

Variance in Structure Within Groups. To display individual differences in volume within groups, we present figures for each area of the brain that we studied, showing each subject's data, by group (Fig. S3).

1. Egger HL, Angold A (2012) The Preschool Age Psychiatric Assessment (PAPA): A structured parent interview for diagnosing psychiatric disorders in preschool children. *Handbook of Infant and Toddler Mental Health Assessment*, eds. Delcarmen-Wiggens R, Carter A (Oxford Univ Press, New York).
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3. Zeanah CH, et al. (2009) Institutional rearing and psychiatric disorders in Romanian preschool children. *Am J Psychiatry* 166:777–785.
4. Ségonne F, et al. (2004) A hybrid approach to the skull stripping problem in MRI. *Neuroimage* 22:1060–1075.
5. Burgund ED, et al. (2002) The feasibility of a common stereotactic space for children and adults in fMRI studies of development. *Neuroimage* 17:184–200.
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8. Preacher KJ, Hayes AF (2004) SPSS and SAS procedures for estimating indirect effects in simple mediation models. *Behav Res Methods Instrum Comput* 36:717–731.
9. Shrout PE, Bolger N (2002) Mediation in experimental and nonexperimental studies: New procedures and recommendations. *Psychol Methods* 7:422–445.

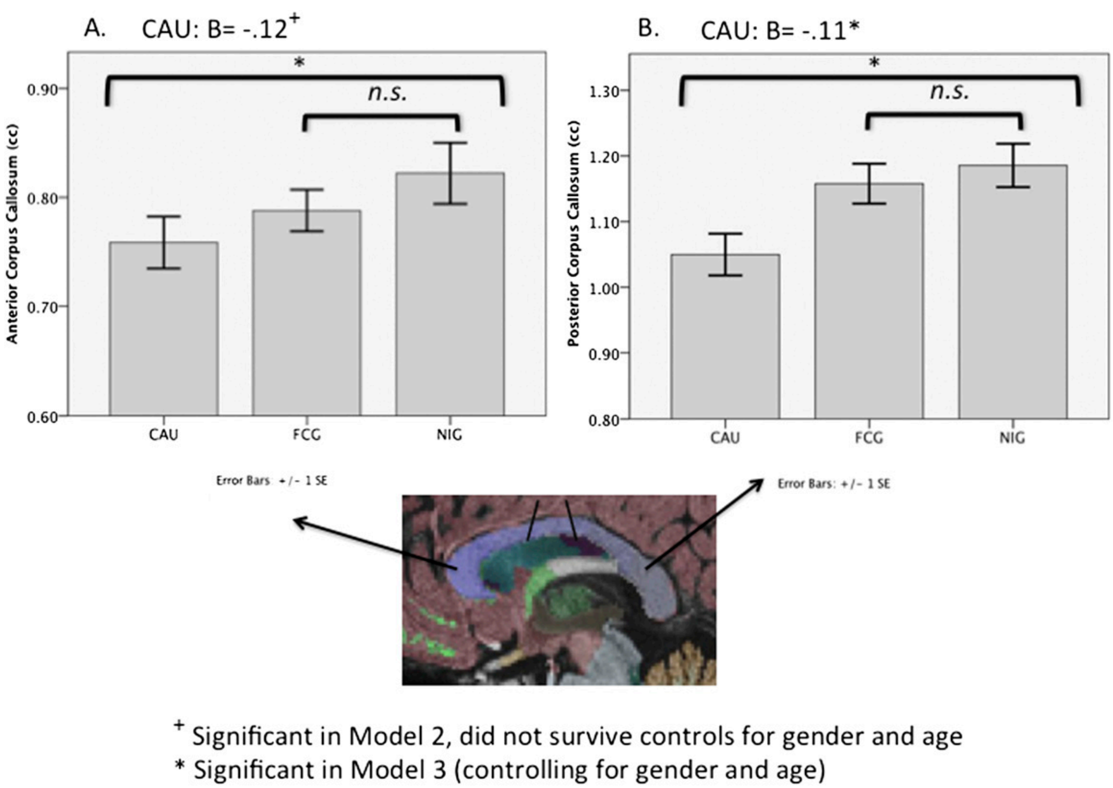

Fig. S1. Average CC volume in cubic centimeters (cm^3) for the anterior (*A*) and posterior (*B*) CC for the CAUG, FCG, and NIG; error bars are ± 1 SEM.

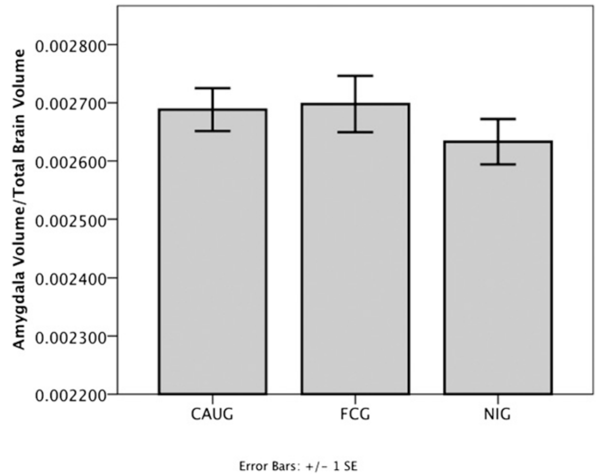

Fig. S2. Average values for the ratio of amygdala/total brain volume for the three groups: CAUG, FCG, and NIG. Differences between the groups are not significant.

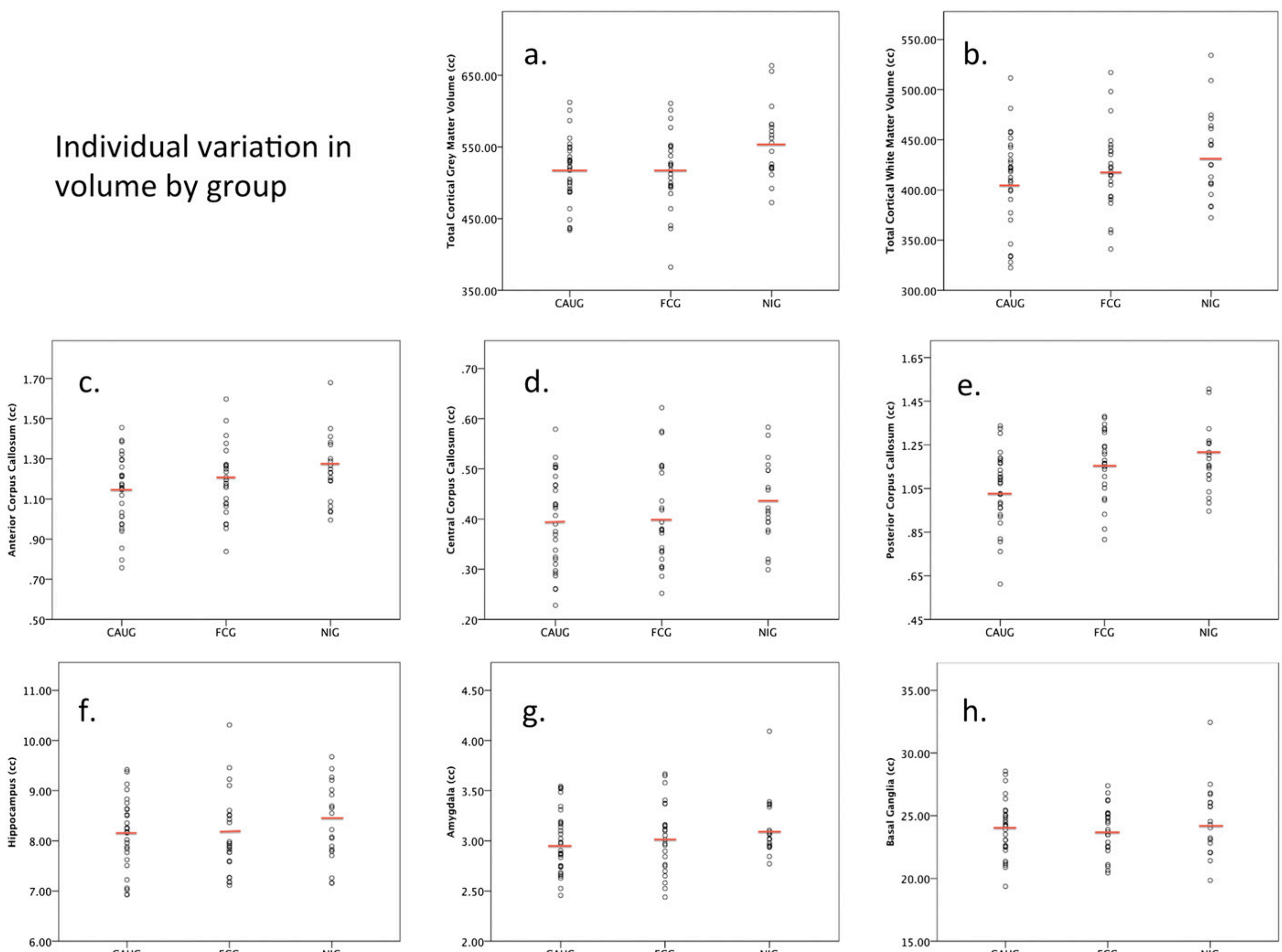

Fig. S3. Scatter plots showing individual variation within group for volume of each structure measured: (*A*) total cortical gray matter, (*B*) total cortical white matter, (*C*) anterior CC, (*D*) central CC, (*E*) posterior CC, (*F*) hippocampus, (*G*) amygdala, and (*H*) basal ganglia. Each group is plotted separately: CAUG, FCG, and NIG. Red lines indicate approximate location of the mean for each group for each structure. For exact means, see Table S2. For some structures, apparent outliers exist (see e.g., *G*: amygdala volume, NIG). In these cases, analyses were completed with and without the outlier; results did not differ.

Table S1. Characteristics of institutionalized children randomized to the foster care intervention or usual care

	Foster care (n = 25)	Care as usual (n = 29)	Never institutionalized (n = 20)
Female, no. (%)	11 (44%)	16 (55%)	10 (50%)
Age at MRI, mean (SD) years	9.92 (0.62)	9.68 (0.79)	9.63 (0.83)
IQ (SD)	64.9 (19.1)	73.6 (16.2)	104.05 (17.03)

Table S2. Average volumes and SDs of all measured structures by group

Structure	Foster care (n = 25)	Care as usual (n = 29)	Never institutionalized (n = 20)
Total gray matter volume (SD)	515.38 (53.5)	514.72 (46.3)	550.43 (53.5)
Total white matter volume (SD)	421.59 (40.8)	407.23 (48.1)	434.9 (42.4)
Posterior CC (SD)	1.15 (0.15)	1.04 (0.17)	1.19 (0.15)
Central CC (SD)	0.40 (0.10)	0.39 (0.09)	0.44 (0.08)
Anterior CC (SD)	1.19 (0.18)	1.14 (0.18)	1.25 (0.17)
Basal ganglia (SD)	23.96 (1.92)	24.03 (2.23)	24.53 (2.77)
Amygdala (SD)	3.04 (0.34)	2.99 (0.31)	3.10 (0.29)
Hippocampus (SD)	8.13 (0.79)	8.14 (0.70)	8.3 (0.78)
Thalamus (SD)	14.09 (1.32)	13.95 (1.6)	14.71 (1.46)

All volumes are in cubic centimeters (cm^3).

Table S3. Association between institutionalization and subcortical volume

MRI subcortical volumes	Model 1	Model 2	Model 3 (adjusted for covariates)*
	β (SE)	β (SE)	β (SE)
Hippocampus			
EIG	−0.15 (0.19)		
CAU		−0.16 (0.22)	0.18 (0.17)
FCG		−0.18 (0.23)	0.13 (0.18)
Amygdala			
EIG	−0.08 (0.08)		
CAU		−0.12 (0.09)	−0.01 (0.07)
FCG		−0.06 (0.10)	−0.02 (0.07)
Basal ganglia			
EIG	−0.48 (0.42)		
CAU		−0.49 (0.46)	0.64 (0.45)
FCG		−0.57 (0.41)	0.40 (0.47)

*Covariates are age, sex, and total brain volume.