Decreased Amygdala Reactivity to Parent Cues Protects Against Anxiety Following Early Adversity: An Examination Across 3 Years

Supplemental Information

Supplemental Methods

Participants. Before exclusions, the total sample size was N=109. Participants were then excluded for excessive motion, i.e., >30% censored volumes (Comp=0, PI=1), $X^2(1)=1.38$, $p=.240$; imaging outliers (>3SD from the mean amygdala signal; Comp=3, PI=0), $X^2(1)=2.12$, $p=.269$; or amygdala coverage < 60% (Comp=3, PI=0), $X^2(1)=2.12$, $p=.269$, leaving a final sample size of N=102 (see supplemental figure S1 for a flow chart of sample size throughout the various components of the study).

Although estimated IQ (N=100) fell within the normal range for both groups, scores were higher in Comparisons (Comparison M=112.92 ± 17.93) than in PI youths (M=101.18 ± 15.67, $t(95)=3.4$, $p=.001$). Due to concerns about potentially high prenatal exposure to alcohol in the PI group and its effects on amygdala reactivity, we examined group differences in fetal alcohol spectrum (FAS) facial characteristics using standardized procedures [1]. We did not see evidence of significant group differences in FAS facial morphology (covarying for age group), $F(1,91)=1.19$, $p=.238$. In the PI group, 3 participants were coded as having elevated FAS-like facial morphology, but this was not associated with amygdala buffering, $X^2(3)=4.59$, $p=.204$, and therefore was not included in subsequent analyses.

In previous studies [2], we have examined cross-sectional data for the comparison sample (87% overlap with current comparison sample). We have also previously published a portion of the cross-sectional data from the PI sample on this task [3] with a different region of interest (ROI) and question of interest.

To examine our hypothesis, that decreased amygdala reactivity to parent cues (which is seen in the Comparison children) would not be evident in the group of children that were previously institutionalized we utilized the same age split as our prior study [2] that reported decreased amygdala reactivity to parent cues in 'children' (<10.5 years), but not in adolescents (>10.5 years) across 'typical' (i.e., no parental deprivation) development. To examine hormonal differences between children and adolescents, we used a univariate ANVOA with the log of salivary testosterone levels at waking as the outcome variable. Salivary testosterone was available in N=82 of the original N=102 youth. We saw a main effect of age group, $F(1,78) = 18.33$, $p<.0001$, whereby adolescents had higher waking testosterone levels than children. The effect of sex, caregiving group, and interaction between Caregiving Group and Age Group were not significant, largest $F=3.91$, $p=.052$.

Questionnaire - Revised child anxiety and depression scale parent version (RCADS-P). Participants with and without follow-up data on the RCADS-P did not differ in terms of caregiving condition, $X^2(2)=2.82$, $p=.244$, age at scan, $t(100)=.63$, $p=.533$, sex, $X^2(2)=.16$, $p=.922$, amygdala reactivity for the parent-stranger difference score, $t(100)=.08$, $p=.923$, average amygdala reactivity for parent: $t(100)=.22$, $p=.827$; or stranger: $t(100)=.25$, $p=.802$, or anxiety scores, $t(100)=.55$, $p=.586$, at Time 1. The same parent that attended the first session (and filled out the first set of questionnaires) was asked to complete the second set of questionnaires.

Total age-adjusted scores above 65 indicate the potential presence of an anxiety disorder. At Time 1, 5% of PI participants (n=2) and 0% of Comp participants scored at or above this cut-off (although continuous scores were significantly higher for the PI group: Comp mean (SD)=12.00 (7.78); PI mean (SD)=24.27 (13.61), $t(97)=-5.33$, $p< .0001$). At Time 2, 6% of participants that were PI (n=2, one of whom remained in the elevated range since Time 1 and another that entered the elevated range between Time 1 and 2) and 0% of

Comp participants (n=0) scored at or above the clinical cut-off (Mean (SD) anxiety scores: Comp=9.07 (6.86); PI=20.97 (15.10), $t(70)=-4.03$, $p=.0003$). As a group, anxiety decreased over time in the Comparisons only, $t(40)=2.80$, $p=.008$, but not in the PI group, $t(28)=1.47$, $p=.152$.

Parent/stranger fMRI task. Participants completed a block-design 'parent/stranger' task while in the scanner. In that task, participants were presented with color photographs of their parent and an ethnicity- and sex-matched unfamiliar individual (who was the parent of another child in the study) in alternating blocks of 28 seconds each. The stimuli were posed with happy and neutral facial expressions on a blank background with white material around their necks and each image was standardized for size and luminance (vertical visual angle of 15°). Thus, for each participant there were two own-parent and two stranger-parent stimuli (happy and neutral, each 50% probability) presented in a fixed random order (vertical visual angle of 15°) over 8 alternating blocks with three blocks of fixation (+PSPS+SPSP+; counterbalanced order). Participants were instructed to respond quickly (within 1500 ms) by pressing a button when they saw a happy facial expression, regardless of the model. Within each block there were 18 parent/stranger stimuli, each presented for 500ms followed by a 1-sec fixation, resulting in 144 total stimuli (72 each of parent and stranger). The entire task lasted for 4.34 minutes. Visual stimuli were presented via MRI-safe video goggles (model: VisuaStim Digital, Resonance Technology, Los Angeles California) and a response pad (model: 932 fORP, Current Designs, Inc., Philadelphia, Pennsylvania) was used to record behavioral responses. Participants were given an opportunity to practice the task before they entered the scanner to ensure that they understood the task instructions and could perform the task.

fMRI preprocessing. Functional imaging data were pre-processed and analyzed with the Analysis of Functional NeuroImages (AFNI, version 16.1.28) software package [4].

Volumes with excessive absolute motion (>.5 voxel from reference volume) were censored. Preprocessing steps were as follows: slice-timing correction, image registration to the first volume, and smoothing with an anisotropic 6mm Gaussian kernel (FWHM). Time series were normalized to percent signal change to allow comparisons across individual subjects by dividing signal intensity at each time point by mean signal intensity for that voxel and multiplying the result by 100. Finally, high-resolution (MPRAGE) structural scans were transformed into Talairach space and then the functional data were moved into Talairach space using each participant's Talairach-transformed MPRAGE scan parameters (resampled resolution of $3mm^3$). Alignment between anatomical and functional scans was assessed visually and any functional data that appeared misaligned were corrected with a rigid body transformation using 6 degrees of freedom. Single subject models included regressors for each stimulus type (parent/stranger) by convolving the stimulus timing files with a canonical hemodynamic response function. Six motion parameters were included as separate regressors, for a total of eight regressors. General linear modeling (GLM; random effects) was performed to fit the percent signal change time courses to each regressor, modeling drift with linear and quadratic factors within each model.

Motion correction. To systematically reduce and control for motion we implemented the following procedures: before the scanning session all participants were acclimated to the scanning environment with a mock scanner. During this practice session, children were given real-time feedback on their motion to optimize their ability to remain still. During data collection, motion was further reduced through the use of head padding to secure the head in a comfortable and steady position. During preprocessing, motion was dealt with by censoring ("scrubbing") volumes with absolute motion >.5 voxel from reference volume. Participants with excessive data loss (>30% of all volumes) were not included.

Behavioral analyses in scanner. We calculated the mean number of 'hits', 'false alarms' and mean reaction time (RT) in each condition (parent and stranger) for each group. For each variable (hit, false alarm, RT) we performed repeated-measures ANCOVA in SPSS using the stimulus type (parent vs. stranger) as the within-subjects variable and with between subject factors of age group (Child vs Adolescent) and caregiving group (Comparison vs. PI). The significance threshold was set at an alpha value of .05 for all analyses.

Assessment of the prospective association between amygdala reactivity to parent and stranger cues and anxiety across time using only participants that contributed data at Time 1 and Time 2. Due to missing data in the longitudinal sample, we aimed to confirm that the Time x Caregiving Group x Amygdala Signal interaction remained significant when including only participants that contributed anxiety data at Time 1 and Time 2 (N = 70; i.e., in a sample with no missing data). We performed the same Mixed Linear Model reported in the main text, but only including those participants with two anxiety data points. Caregiving condition (Comparison vs. PI), age group (child vs. adolescent), sex (male vs. female), time (Time 1 vs. Time 2), and amygdala beta weights were entered as fixed effects predictors of RCADS scores, with random slope and intercept between individuals.

Supplemental Results

In-scanner 'mother/stranger' task performance. Correct hits (to happy), false alarms (to neutral), and reaction times (RT; to happy) were calculated for each subject (N=102 with useable data). Participants that were outliers on any variable (>3SD from the mean value) were excluded from the analysis. Using these criteria, there were 7 outliers excluded (Comparison n=4; PI n=3). Data were analyzed with repeated measures ANOVA, with trial type (parent vs. stranger) as the within-subjects factor (controlling for participant sex). For hits, there was a main effect of trial type, $F(1,90)=4.54$, $p=.036$, $\eta_p^2=.05$, and age

group, $F(1,90)=4.94$, $p=.029$, $\eta_p^2=.052$, whereby more hits were made to parent than stranger faces (parent mean/SD=.95/.06, stranger mean/SD=.94/.08) and more hits were made by adolescents than children (adolescent mean/SD parent=.97/.05, adolescent mean/SD stranger=.96/.08, child mean/SD parent=.94/.07, child mean/SD stranger=.93/.08). No other main effects and interactions were significant, largest $F(1,90)=1.63$, $p=.205$, $\eta_p^2=.02$. For false alarms (FA) there was a significant main effect of trial type, $F(1,90)=19.74$, $p<.001$, $\eta_p^2=.18$, and age group, $F(1,90)=13.00$, $p=.001$, $\eta_p^2=.12$, whereby more FA occurred to pictures of the parent than stranger (parent mean/SD=.19/.14, stranger mean/SD=.14/.14), and more FA were made by children than adolescents (child mean/SD to parent=.24/.14, child mean/SD to stranger=.19/.15, adolescent mean/SD parent=.14/.11, adolescent mean/SD stranger=.10/.12). No other main effects and interactions were significant, largest $F(1,90)=2.17$, $p=.144$, $\eta_p^2=.02$. For reaction times (RTs) there was a main effect of trial type, $F(1,90)=5.45$, $p=.022$, $\eta_p^2=.06$, and age group, $F(1,90)=45.58$, $p<.0001$, whereby RTs (in milliseconds) were faster to pictures of the parent than the stranger (parent mean/SD RT=448/105, stranger mean/SD RT=420/129), and RTs in adolescents were faster than RTs in children (child mean/SD RT parent= 498/101, child mean/SD RT stranger=486/119, adolescent mean/SD RT parent=398/84, adolescent mean/SD RT stranger=355/104). No other main effects and interactions were significant, largest $F(1,90)=3.17$, $p=.079$, $\eta_p^2=.03$.

RCADS – total summed anxiety. As anticipated because of the nature of recruitment, PI children had higher scores than Comparison children on the RCADS at baseline $t(47)=4.49$, $p<.0001$, $d=.1.29$, and at follow-up, $t(33)=2.80$, $p=.012$, $d=1.02$. PI adolescents also had higher RCADS scores than Comparison adolescents at baseline, $t(45)=3.00$, $p=.004$, $\eta^2_p=.89$, and at follow-up, $t(35)=3.23$, $p=.003$, $d=1.09$.

Right amygdala reactivity – Bayesian post hoc tests of the difference between mother and stranger for each group. We used Bayesian analysis (1-sided, one sample t-

test, JASP Team, 2018) to test whether the difference in amygdala reactivity to parent vs. stranger was more likely to fall under the null hypothesis (i.e., no difference in amygdala response to parent vs. stranger pictures) than under the alternative hypothesis (i.e., lower amygdala reactivity to parent relative to stranger pictures) independently for each group. Analyses for PI children were reported in the main text. For the Comp Children, the Bayes Factor indicated the data were 1.69 times more likely to be observed under the alternative, than null hypothesis. For PI and Comp adolescents, the Bayes Factor indicated the data were 3.63, and 13.29 times more likely (respectively) to be observed under the null than alternative hypothesis.

Right amygdala reactivity – age continuous. When age was treated continuously there was no main effect of age, $F(10, 63)=.1.10$, $p=.374$, $\eta^2_p=.15$, or caregiving group, $F(1,63)=.50$, $p=.483$, $\eta^2_p=.01$, and no Age by Caregiving Group interaction, $F(10,63)=1.39$, $p=.204$, $\eta^2_p=.18$, on right amygdala signal.

Amygdala reactivity left amygdala and sub-region analysis. Although we had *a priori* hypotheses about right amygdala reactivity to parent cues, for completeness we also examined left amygdala signal for evidence of decreased reactivity to parent cues. There was no effect of age group, $F(1,93)=2.62$, $p=.109$, $\eta^2_p=.03$, or caregiving group, $F(1,93)=2.00$, $p=.160$, $\eta^2_p=.02$, but the Caregiving Group by Age Group interaction was significant, $F(1,93)=6.29$, $p=.014$, $\eta^2_p=.06$. Importantly, however, decreased reactivity to parent cues in the left amygdala was not predictive of future anxiety scores. Unlike the right amygdala, there was no Caregiving Group x Left Amygdala Signal x Time interaction, $F(1,72.63)=1.02$, $p=.315$, which is consistent with our hypothesis of the importance of decreased reactivity to parent cues in the right amygdala for future emotion regulation.

In addition to the left whole amygdala, we also interrogated each amygdala subdivision for evidence of decreased reactivity to parent cues. There was a significant Age

Group by Caregiving Group interaction in the left superficial amygdala (SF), $F(1,93)=7.26$, $p=.008$, $\eta^2_p=.07$, left latero basal amygdala (LB), $F(1,93)=6.63$, $p=.012$, $\eta^2_p=.06$, left central medial amygdala (CM), $F(1,93)=4.45$, $p=.038$, $\eta^2_p=.05$, and right LB, $F(1,93)=5.16$, $p=.025$, $\eta^2_p=.05$, but not in the right SF, $F(1,93)=3.90$, $p=.051$, $\eta^2_p=.04$, and right CM, $F(1,93)=2.89$, $p=.092$, $\eta^2_p=.03$. Regardless of the statistical outcome, the pattern of amygdala reactivity was consistent for every interaction, whereby lower amygdala reactivity to parent than stranger cues was observed in the comparison children only, and all other groups exhibited higher amygdala reactivity to parent than stranger stimuli.

Right amygdala decreases to parent cues of as a predictor of future anxiety – full report of model results. In addition to the Time x Caregiving Group x Amygdala Signal interaction reported on in the main text, the following main effects and interactions were significant in the mixed linear model (N=101): there were significant main effects of Caregiving Group, $F(1,100.87)=28.9$, $p<.0001$, $\eta^2_p=.22$, and Time, $F(1,75.33)=18.48$, $p<.0001$, $\eta^2_p=.20$. There was a significant interaction between Caregiving Group x Sex x Time, $F(1,75.54)=4.12$, $p=.046$, $\eta^2_p=.05$, Sex x Age Group x Time, $F(1,75.53)=14.41$, $p=.0003$, $\eta^2_p=.16$, Sex x Time x Amygdala Signal, $F(1,75.65)=4.44$, $p=.038$, $\eta^2_p=.05$, Sex x Age Groups x Time x Amygdala Signal, $F(1,75.24)=5.66$, $p=.020$, $\eta^2_p=.07$, and Caregiving Group x Sex x Age Group x Time, $F(1,75.76)=22.41$, $p<.0001$, $\eta^2_p=.23$. Post-hoc tests revealed that the sex interactions were driven by PI males exhibiting lower anxiety scores than PI females at the follow-up assessment, $F(1,96.76)=5.2$, $p=.025$, $\eta^2_p=.05$, by PI male children exhibiting lower anxiety scores than PI female children at follow-up, $F(1,95.60)=10.30$, $p=.002$, $\eta^2_p=.09$, and by PI male adolescents exhibiting lower anxiety scores than PI female adolescents at baseline, $F(1,99.97)=4.19$, $p=.043$, $\eta^2_p=.04$.

For the post hoc analyses we saw that although Time 1 anxiety symptoms were higher in the PI group than in the Comparison group (both for individuals that had lower amygdala reactivity to the parent - 'lower to parent': $F(1,99.89)=16.14$, $p<.0001$, $\eta^2_p=.14$, and for those that did not have lower amygdala to parent – 'not lower to parent': $F(1,101.69)=19.13$, $p<.0001$, $\eta^2_p=.16$), at Time 2, group differences in anxiety symptoms only remained in individuals that did not show amygdala decreases to parent cues (i.e., in the 'not lower to parent' individuals), $F(1,92.95)=22.62$, $p<.0001$, $\eta^2_p=.20$. For the youth that exhibited amygdala decreases to parent cues, there were no group differences in anxiety scores between Comparison and PI individuals at Time 2, $F(1,94.57)=3.12$, $p=.081$, $\eta^2_p=.03$.

Right amygdala decreases to parent cues as a predictor of future anxiety – limited dataset analysis with participants contributing both Time 1 and Time 2 anxiety data points. The Time x Caregiving Group x Amygdala Signal interaction remained significant in this analysis, $F(1,70)=7.70$, $p=.007$, $\eta^2_p=.10$, suggesting that the model reported in the main text was equipped to deal with the missing data points in our sample.

Association between amygdala decreases to parent cues and attachment security in comparison youth. Child/adolescent reported attachment security with the biological parent did not predict whether amygdala buffering occurred in comparison youth, $\beta=.05$, $t(46)=.40$, $p=.691$, $d=.15$.

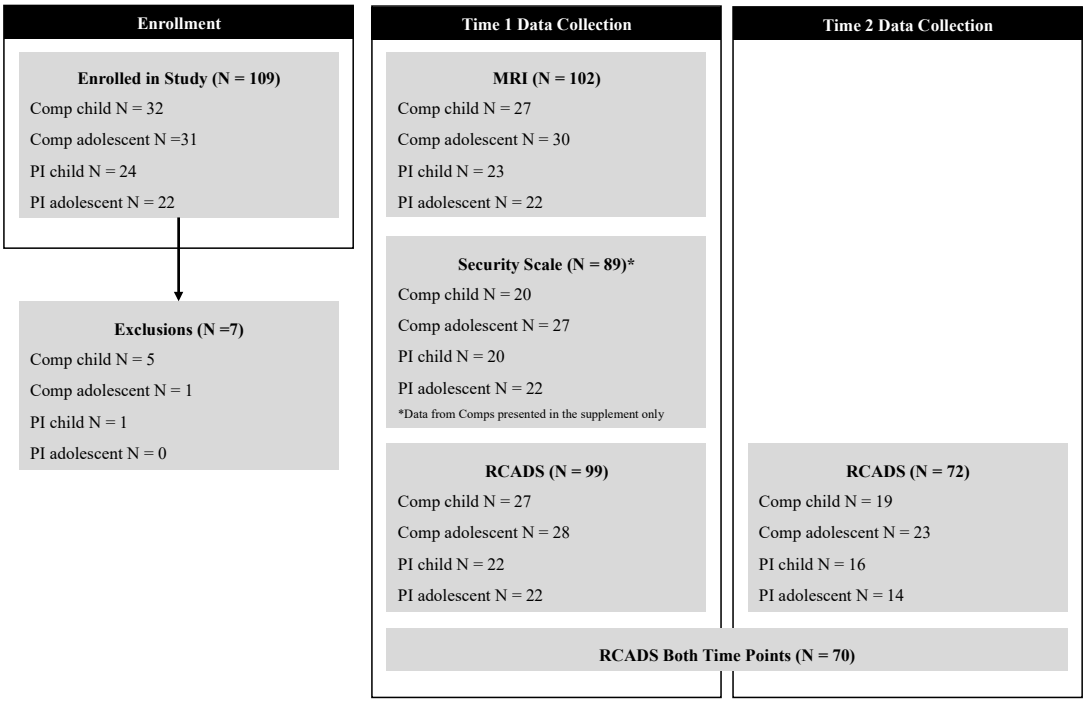

Figure S1. Flow chart of the sample size (and exclusions) throughout the different stages of the study.

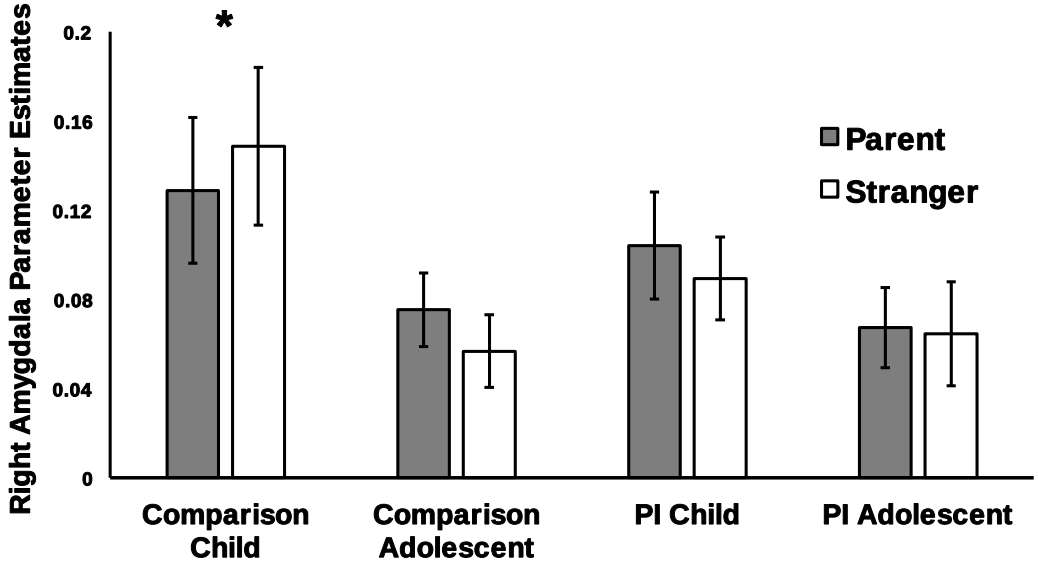

Figure S2. The graph shows the mean extracted β weights from the right amygdala in the parent (grey bars) and stranger (white bars) condition across both caregiving groups (Comparison and PI) and age groups (children and adolescents). The single asterisk (*) indicates a significant within-subject difference between the two conditions. Error bars show ±1 *SEM*. subject (Montreal Neurological Institute coordinate y=-4; R=right; L=left).

Table S1. Demographic Information

	Comp Children (n = 27)	PI Children (n = 23)	p-value for difference between children	Comp Adolescents (n = 30)	PI Adolescents (n = 22)	p-value for difference between adolescents
Mean age in years at scan – Time 1 (SD; Range)	7.73 (1.57; 5-10)	8.44 (1.31; 6-10)	.095	13.39 (1.86; 10-16)	13.13 (1.74; 10-16)	.595
Mean age in years at follow-up – Time 2 (SD; range)	9.98 (1.83; 7-14)	10.48 (1.36; 8-13)	.372	15.39 (2.08; 11-19)	15.43 (1.49; 13-18)	.958
Mean age in years at follow-up – Time 3 (SD; range)	11.11 (1.32; 8-13)	11.91 (1.66; 9-14)	.191	16.53 (1.98; 13-20)	16.79 (1.32; 15-18)	.717
Mean months of age when entered institution (SD; range)	–	6.85 (11.29; 0-36)	–	–	10.62 (19.39; 0-72)	–
Mean months of age at adoption (SD; range)	–	30.16[a] (27.43; 8-96)	–	–	38.91 (34.18; 6-120)	–
Mean months in institution (SD; range)	–	23[a] (20.89; 7.75-90)	–	–	28.29 (20.46; 5-72)	–
Mean months with adoptive parents by time of scan (SD; range)	–	70.48[a] (28.6; 12-114)	–	–	118.59 (45.21; 19-187)	–
Sex composition of sample (% male)	48%	43%	.741[c]	57%	36%	.171[c]
Mean full-scale IQ (SD; range)	123.25 (16.95; 84-149)	102.64[a] (17.63; 69-133)	.0002	104.38 [a] (13.92; 76-129)	99.73 (13.70; 72-123)	.240

Comp, comparison group; PI, previously institutionalized group.
[a]n = 1 missing data
[b]n = 3 missing data
[c]Chi-square analysis

Table S2. Racial, income and education information in Comparison and PI youths.

	% Comp Children	% Comp Adolescents^^	% PI Children	% PI Adolescents
Racial Background of Youths^				
European American	67	43	61	50
African American	26	47	0	5
Asian American	37	10	26	36
American Indian	0	10	0	0
Other American	0	0	13	9
Cultural Identification				
Hispanic	7	10	0	5
Family Demographics				
Annual Modal Household Income**	+$200,000 USD	+$200,000 USD	$100,000-150,000 USD	$150,000-200,000 USD
Primary Caregiver Highest Modal Education***	4 year college degree	4 year college degree	4 year college degree	4 year college degree
Region of Origin PI Youths*				
Eastern Europe	-	-	48	36
East and South Asia	-	-	48	55
Middle East	-	-	4.5	4.5
Africa	-	-	0	4.5

^participants could belong to more than one ethnic or racial group.
^^n = 1 participant missing data from the comparison adolescent group.
*n = 5 participants with missing data from previously institutionalized child group and 8 participants with missing data from the previously institutionalized adolescent group.
**Data on household income was obtained from 96 families (comparison n = 53; PI n = 43)
***Data on primary caregiver education was obtained from 100 families (Comparison n = 56; PI n = 43).

Table S3. Extracted amygdala betas for the left whole amygdala and for each sub-region from the right and left amygdala separately in previously institutionalized (PI) and comparison (COMP) children and adolescents.

	Child COMP (N=27)	Child PI (N=23)	Adolescent COMP (N=30)	Adolescent PI (N=22)
Left Whole Amygdala Mean, (*SD*)	-0.029, (*0.100)*	0.240, (*0.062*)	0.029, (*0.047*)	0.010, (*0.058*)
Left SF Mean, (*SD*)	-0.028, (*0.089*)	0.029, (*0.090*)	0.028, (*0.056*)	0.007, (*0.051*)
Left CM Mean, (*SD*)	-0.014, (*0.112*)	0.028, (0.064)	0.034, (0.048)	0.014, (*0.048*)
Left LB Mean, (*SD*)	-0.030, (*0.075*)	0.021, (0.061)	0.016, (0.036)	0.009, (*0.053*)
Right SF Mean, (*SD*)	-0.016, (0.080)	0.020, (*0.085*)	0.020, (0.036)	0.007, (0.054)
Right CM Mean, (*SD*)	-0.007, (*0.071*)	0.009, (*0.075*)	0.030, (0.042)	0.009, (0.040)
Right LB Mean, (*SD*)	-0.014, (*0.045*)	0.015, (*0.073*)	0.006, (*0.042*)	-0.003, (*0.045*)

Superficial Amygdala (SF), Central Amygdala (CM), Lateral Basal (LB).

Supplemental References

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