NEW RESEARCH

A Randomized Controlled Trial of a Parenting Intervention During Infancy Alters Amygdala-Prefrontal Circuitry in Middle Childhood

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Objective: Early adverse parenting predicts various negative outcomes, including psychopathology and altered development. Animal work suggests that adverse parenting might change amygdala–prefrontal cortex (PFC) circuitry, but work in humans remains correlational. The present study leveraged data from a randomized controlled trial examining the efficacy of an early parenting intervention targeting parental nurturance and sensitivity (Attachment and Biobehavioral Catch-up [ABC]) to test whether early parenting quality causally affects amygdala-PFC connectivity later in life.

Method: Participants (N = 60, mean age = 10.0 years) included 41 high-risk children whose parents were referred by Child Protective Services and randomly assigned to receive either ABC (n = 21) or a control intervention (n = 20) during the children's infancy and a comparison sample of low-risk children (n = 19). Amygdala-PFC connectivity was assessed via functional magnetic resonance imaging while children viewed fearful and neutral faces.

Results: Across facial expressions, ABC produced different changes than the control intervention in amygdala-PFC connectivity in response to faces. The ABC group also exhibited greater responses than the control intervention group to faces in areas classically associated with emotion regulation, including the orbitofrontal cortex and right insula. Mediation analysis suggested that the effect of ABC on PFC activation was mediated by the intervention's effect on amygdala-PFC connectivity.

Conclusion: Results provide preliminary causal evidence for the effect of early parenting intervention on amygdala-PFC connectivity and on PFC responses to face viewing. Findings also highlight amygdala-PFC connectivity as a potential mediator of the effects of early parenting intervention on children's emotion regulation development.

Clinical trial registration information: Intervening Early With Neglected Children; https://clinicaltrials.gov/; NCT02093052.

Diversity & Inclusion Statement: We worked to ensure sex and gender balance in the recruitment of human participants. We worked to ensure race, ethnic, and/or other types of diversity in the recruitment of human participants. We worked to ensure that the study questionnaires were prepared in an inclusive way. One or more of the authors of this paper self-identifies as a member of one or more historically underrepresented racial and/or ethnic groups in science. One or more of the authors of this paper self-identifies as a member of one or more historically underrepresented sexual and/or gender groups in science. One or more of the authors of this paper received support from a program designed to increase minority representation in science. While citing references scientifically relevant for this work, we also actively worked to promote sex and gender balance in our reference list.

Key words: amygdala; early adversity; parenting; prefrontal cortex; randomized controlled trial

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Parenting quality, especially during early life, influences development.[1] Adverse parenting, such as childhood maltreatment and neglect, has been linked to many negative outcomes, including psychopathology and altered development.[2,3] The amygdala, through its abundant connections with the prefrontal cortex (PFC), might mediate relations between early parenting and emotional development.[4,5] Causal evidence for this amygdala-mediated parenting pathway exists in nonhuman animals[6]; however, in humans, this work remains correlational.[4] To test whether early-life changes in parenting affect later-life amygdala connectivity and PFC responses to emotional stimuli, the present study leveraged data from a randomized controlled trial (RCT) examining the efficacy of an early parenting intervention.

Studies in rodents and nonhuman primates suggest that early parenting impacts amygdala-PFC circuitry. This experimental work causally links parenting quality to aspects of amygdala development, including premature amygdala activation during avoidance and fear learning,[7,8] early growth and myelination of amygdala neurons,[9] enhanced amygdala excitability,[6] and altered amygdala-

PFC connectivity and plasticity.[10,11] Similarly, work in humans links adverse early parenting to amygdala development and amygdala-PFC connectivity.[12-19] However, this work in humans remains observational.

Early interventions afford an opportunity to causally link parenting to human brain development. Parenting interventions have been shown to increase parental responsiveness and nurturance, thereby improving attachment quality and physiological and behavioral regulation of infants.[20-25] One such early parenting intervention is Attachment and Biobehavioral Catch-up (ABC).[26] ABC is delivered in the home by trained parent coaches across 10 sessions. It increases rates of secure attachment and improves biological and behavioral regulation of children by enhancing parental nurturance when children are distressed, enhancing parental sensitivity when children are not distressed, and decreasing frightening and intrusive behaviors.[26] Together, these changes to parenting behaviors are thought to increase the parent's physical and psychological availability to the child, thus providing an effective coregulator of potentially overwhelming emotions. Gradually, as the child's cognitive abilities develop and with continued sensitive support from the parent across early childhood, the child is increasingly able to regulate their emotions independently.[27,28]

The efficacy of ABC has been established through multiple RCTs involving vulnerable populations, including children in the foster care system,[27] children living with birth parents following involvement with Child Protective Services (CPS),[29] and children who were adopted internationally.[30] Parents randomly assigned to receive ABC demonstrate greater sensitivity and positive regard and lower intrusiveness and withdrawal than parents who received a control intervention.[30] Children of parents who received ABC vs a control intervention exhibit improvements in several indicators linked to emotion regulation, including attachment,[23,30] autonomic regulation,[31] cortisol rhythms,[32] and executive functioning skills.[27,29] A recent neuroimaging follow-up study by our group examined brain responses of 8- to 12-year-old children of parents who received either ABC or a control intervention while the children were infants. Children from the ABC group showed greater activation to maternal cues in clusters of brain regions including the precuneus and posterior cingulate cortex, and greater activity in these brain regions explained the effect of the ABC intervention on improved behavior problems in children relative to a control group.[33]

Given previous work linking ABC to improved emotion regulation, the current study probed, via an RCT, the impact of ABC on neurobiological functioning that has been widely associated with emotion regulation, namely, amygdala and PFC functioning and connectivity. Children completed an emotional face viewing functional magnetic resonance imaging (fMRI) task when they were 8 to 12 years old. First, because past work has shown that ABC may improve emotion regulation skills in children,[28] we hypothesized that children from the ABC group would show greater PFC activation than children from the control intervention group in response to emotional faces, in line with the idea that use of emotion regulation strategies is associated with greater PFC recruitment.[34] Second, we hypothesized that any intervention group differences in PFC activation would be explained by differences in amygdala-PFC connectivity, as amygdala-PFC connectivity may mediate the link between the early parenting context and changes in PFC function.[4]

METHOD

Participants

Families (N = 212) were originally recruited as part of an RCT (ClinicalTrials.gov identifier: NCT02093052) when children were infants in a major Mid-Atlantic city. As part of a city-wide initiative designed to redirect children from foster care, families were referred from CPS due to risk for abuse or neglect. Children in this high-risk sample were not necessarily abused or neglected, but were deemed to be at risk for such by CPS due to factors such as homelessness or exposure to domestic violence. Detailed CPS referral information was not available to research staff. On recruitment, enrolled families were randomly assigned to receive either ABC or a control intervention (see CONSORT diagram in Figure S1, available online). Families were unaware of their intervention group assignments. Before intervention, children across the intervention groups did not differ in age, race, or diurnal cortisol levels,[32] and parents did not differ in age, educational attainment, race,[23] parental sensitivity, or attachment-related representations,[31] indicating that randomization was successful and supporting the ability to make causal inferences from intervention group differences. Of the 212 families enrolled in the RCT, 183 participated in initial postintervention follow-up assessments, and 112 participated in 8-year follow-up assessments (Figure S1, available online). A subset of families who participated in the 8-year follow-up assessments were invited to participate in this fMRI substudy. To maximize chances of successful scans, children who successfully completed an EEG assessment as part of an 8-year follow-up visit were invited to participate in this fMRI substudy. This approach was based on the assumption that children who were uncomfortable with a noninvasive EEG cap would likely also be uncomfortable in the cramped MRI

environment. Eligible families were invited to participate while they were in the laboratory for one of the follow-up visits of the larger study. Recruitment for the fMRI substudy ended after a predetermined number of participants completed the fMRI protocol (see below). Ultimately, 54 high-risk children (ABC: n = 27; DEF: n = 27) 8.1 to 12.1 years of age participated in this fMRI substudy. In the scanning sample, there were no significant group differences in demographic variables, including age at scanning (all *ps* > .05) (see Table S1, available online, for demographics).

For comparison with the 2 high-risk groups (ie, the ABC intervention group and the control intervention group), a new sample of 83 children who were not referred by the CPS and did not receive any intervention was recruited at age 8 through local community centers and schools. This sample was matched to the CPS-referred sample on race and sex. Families were ineligible for recruitment to the low-risk sample if they had any history of CPS involvement. As in the high-risk sample, comparison children who completed the 8-year EEG assessment were subsequently invited to participate in this fMRI substudy. The fMRI low-risk comparison sample consisted of 26 children 9.1 to 11.0 years of age. Recruitment for the fMRI substudy ended after a total of 80 children participated in the fMRI substudy as predetermined (ABC: n = 27; DEF: n = 27; low-risk: n = 26).

Experimental Intervention. ABC is a brief (10-session) home-based parenting intervention that promotes sensitive parenting. ABC focuses on 3 main behavioral targets for parents: 1) increasing sensitivity to child signals, 2) increasing nurturance to child distress, and 3) decreasing frightening and harsh behaviors. In addition to manualized content, intervention sessions consist of parent coaches providing in the moment commenting and feedback to support parents in identifying their children's signals and providing responsive care.[26]

Control Intervention. Developmental Education for Families (DEF) is an adaptation of existing interventions[35] that have been shown to promote development of children's motor skills, cognition, and language abilities. Components of the intervention related to parental sensitivity were removed for this study to avoid overlap with ABC.

Procedure

After parents provided informed consent and children provided assent, the children were acclimatized to the scanner using an MRI replica before the scanning session, which typically occurred within 2 weeks of the practice session. The protocol was approved by the Institutional Review Board of the University of Delaware. Parents completed the Child Behavior Checklist as part of a battery of measures (see Supplement 1, available online, for additional details and results).

Imaging

Emotional Face Task. The emotional face viewing task was administered to 73 children (ABC: n = 24; DEF: n = 24; low-risk: n = 25) in the scanner. The block-design task presented gray scale fearful and neutral faces from the NimStim set of facial expressions[36] in alternating blocks. Stimuli included male and female faces from Black, White, and Asian models, each of whom was represented in both the fear and the neutral conditions. Each block lasted 26 seconds and included either 16 fearful faces or 16 neutral faces in a fixed random order. The order of blocks was counterbalanced across participants. Each face was presented for 500 ms and separated by a 900-ms fixation cross. To ensure attention to the task, each block included 2 images of a cartoon butterfly presented for 500 ms, which were randomly interspersed among the face stimuli. Participants were instructed to press a button whenever they saw the butterfly. Accuracy in response to the butterfly images was high (mean [SD] = 90.3% [7.3%]).

Image Acquisition. Images were acquired with a Siemens Prisma 3T MRI scanner (Siemens GmbH, Erlangen, Germany), equipped with a 20-channel head coil. A whole-brain, high-resolution, T1-weighted anatomical scan (magnetization-prepared rapid acquisition gradient-echo; 256 × 256 in-plane resolution, 256-mm field of view, 192 × 1-mm sagittal slices) was used for transformation and localization of each participant's functional data into Montreal Neurological Institute 152 (MNI152) space. For the emotional face task, T2*-weighted echo-planar images (34 slices) were acquired using an oblique angle of approximately 30° from each participant's position, 4-mm slice thickness (skip = 0), repetition time 2000 ms, echo time 30 ms, flip 90°, and matrix 64 × 64.

fMRI Preprocessing. Functional imaging data were preprocessed and analyzed with the FSL version 6.0.1 software package. Preprocessing, single-subject statistics, and higher-level analyses were performed using FSL FEAT. Preprocessing steps included slice-timing correction, motion correction (with FSL MCFLIRT), image registration to the first volume, smoothing with an anisotropic 6-mm Gaussian kernel (full width at half maximum), time series normalization, and transformation into MNI152 space. The regression model included 8 explanatory variables (6 motion parameters and the 2 stimulus types: fear and neutral).

Volumes with excessive framewise motion (>0.9 mm from adjacent volume) were censored, and participants with >30% total volumes censored were excluded from analysis. Three participants from the ABC group, 4 from the DEF group, and 6 from the low-risk group were excluded from analyses due to either excessive motion during the task or to excessive motion during the anatomical scan (which prevented registration of functional imaging data) (see CONSORT diagram in Figure S1, available online, for further exclusion details for the 2 RCT groups). The final sample consisted of 60 children (ABC: n = 21; DEF: n = 20; low-risk: n = 19) included in analyses. There were no significant group differences in age ($F_{2,57} = 0.327$, $p = .72$) or sex ($\chi^2_{2,\ N\ =\ 60} = 0.681$, $p = .71$) in the final sample.

Functional Connectivity. Generalized psychophysiological interaction (gPPI) analyses[37] were conducted to examine potential group differences in task-dependent functional connectivity. Although gPPI may be especially sensitive to preprocessing pipeline choice when used with event-related task designs, gPPI is robust to pipeline choice when applied to block designs such as that used in the current study.[38,39] All gPPI analyses were performed using FEAT with regressors for stimulus type, seed region time series, interaction of stimulus type and time series, and 6 motion regressors. The first gPPI analysis examined amygdala connectivity. A bilateral amygdala mask was defined based on the Harvard-Oxford subcortical structural atlas. This analysis tested for group differences in the extent to which amygdala activity covaried with other brain regions during face processing.

Statistical Analysis

Whole-brain analyses were performed to test the within-subject effects of stimulus type (in the case of gross activation) and of the interaction of stimulus type and seed time series (in the case of functional connectivity) on activity in cortical and subcortical brain regions. Group differences in these effects were tested via a series of planned comparisons. The FLAME 1 mixed effects model was used with the automatic outlier deweighting option. Clusters of blood oxygen level–dependent (BOLD) activation were initially considered significant if $z > 2.3$ with a corrected cluster significance threshold of $p = .05$. In addition, due to the number of group comparisons, the familywise error rate was controlled with the FSL randomise function with threshold-free cluster enhancement, which estimates voxelwise p values for the whole brain as a function of the design matrix, spatial neighborhood information, and four-dimensional BOLD data—all without relying on arbitrary thresholds.[40,41] Six pairwise group contrasts were modeled via the FEAT design matrix (eg, ABC > DEF, DEF > ABC, ABC > Low-Risk) plus 2 contrasts that collapsed across the 2 high-risk groups (ie, High-Risk > Low-Risk and Low-Risk > High-Risk). Given the present causal hypotheses, the current report focuses on results from the ABC > DEF and DEF > ABC contrasts, as these were the only 2 groups to which participants were randomly assigned. Brain structure labels were estimated probabilistically using the Harvard-Oxford cortical and subcortical structural atlases in FSL using the automatic atlas query function autoaq. Lastly, causal mediation analysis[42] was performed in R version 4.2.0 using the mediation package[43] to test whether the effect of ABC on BOLD reactivity to faces was explained by the effect of ABC on amygdala-seeded connectivity. Unstandardized indirect effects were computed for each of 10,000 bootstrapped samples, and 95% CIs were computed by determining the indirect effects at the 2.5th and 97.5th percentiles.

RESULTS

BOLD Activation

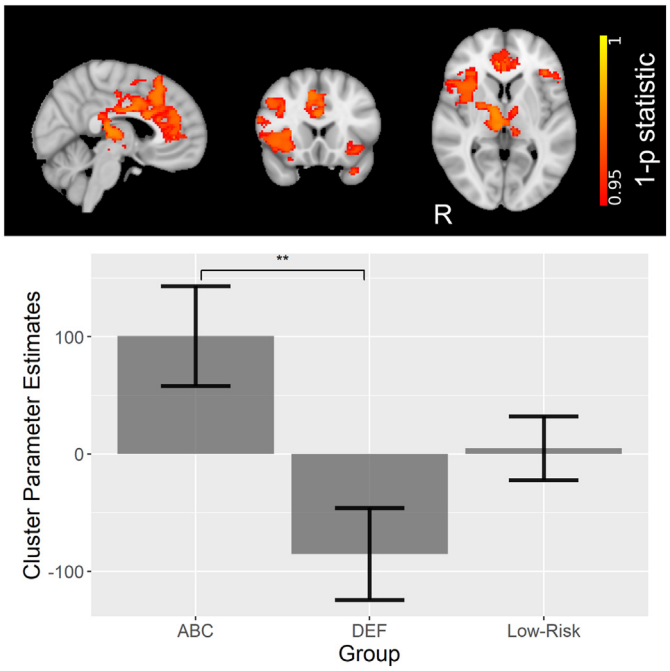
Across groups, fearful faces elicited greater activation than neutral faces in clusters of brain regions including the bilateral amygdala, frontal orbital cortex, temporal fusiform cortex, and occipital cortex (clusterwise $p < .001$) (Figure S2, available online).[12,44] There were no significant clusters where neutral faces were associated with greater activation than fearful faces.

Although there were significant intervention effects when examining BOLD responses to fearful or neutral faces individually (uncorrected clusterwise ps < .04, randomise-corrected ps < .05) (Figure S3, available online), there were no significant between-group differences in fear minus neutral or neutral minus fear contrasts. Therefore, the fearful and neutral face blocks were combined (via an any face vs blank screen stimulus contrast) for subsequent between-group analyses. Across fearful and neutral faces, the ABC group exhibited greater BOLD activation than the DEF group in clusters of brain regions including the anterior cingulate cortex, right orbitofrontal cortex, and right insula (randomise-corrected $p < .05$) (Figure 1, Table 1). Post hoc t tests of these BOLD values revealed that although the ABC and DEF groups significantly differed from each other ($p = .003$), neither intervention group significantly differed from the low-risk group (ps > .05).

BOLD Functional Connectivity

Amygdala Connectivity. There was a significant intervention effect on amygdala-seeded functional connectivity

FIGURE 1 Intervention Effect on Reactivity to Faces



Note: *Colored regions indicate statistically significant clusters where experimental intervention (Attachment and Biobehavioral Catch-up [ABC]) > control intervention (Developmental Education for Families [DEF]) after correction for multiple comparisons. There were no significant clusters where DEF > ABC. Montreal Neurological Institute coordinates x = 5, y = 18, z = 4. Error bars indicate ± 1 SE. Low-risk group parameter estimates are shown in bar graph for comparison. R = right. Please note color figures are available online.*
**p < .01.

while viewing the fearful and neutral faces (uncorrected clusterwise *p*s < .03, randomise-corrected *p*s < .05). Whereas the DEF group showed positive connectivity between the amygdala seed and a cluster of brain regions including the right insula and right frontal orbital cortex, the ABC group instead showed negative connectivity between the amygdala and these areas (Figure 2 and Table 2). Post hoc t tests confirmed that connectivity estimates of both intervention groups were significantly different from zero (*p*s < .04), significantly differed from each other (*p* < .001), and were significantly different from those of the low-risk group (though in opposite directions; *p*s < .02). The same pattern of group differences was observed when using a bilateral dorsal amygdala seed or when using the left and right amygdala as separate seed regions (Figures S4-S6, available online).

Next, we tested the hypothesis that a hierarchical relation between amygdala and PFC exists, such that amygdala changes mediate the observed environment-PFC association.[4,5] A mediation model was fit to test whether the effect of the ABC intervention on BOLD reactivity to faces was explained by the effect of ABC on amygdala-seeded connectivity (Figure 3, left side).[42,43] This model included only the ABC and DEF groups, with intervention group assignment as the predictor, the intervention effect on amygdala-seeded connectivity as the mediator, and the intervention effect on BOLD activation (Figure 1) as the outcome. For mediation analyses, each MRI variable consisted of that participant's average β weights from the significant intervention effect cluster. There was a significant indirect effect via amygdala connectivity (estimate = 130.00, 95% CI [5.90, 265.16], $p = .036$). However, the direct effect of intervention on BOLD activation was no longer significant (estimate = 55.99, 95% CI [−47.55, 178.02], $p = .266$). Approximately 69.9% of the intervention's effect on BOLD activation was explained by amygdala-seeded connectivity.

Because both the mediator and the outcome variables were measured during the same assessment, an alternative mediation model was tested in which the mediator and outcome variables were swapped (Figure 3, right side). Intervention group remained the predictor, but in this model, BOLD activation served as the mediator, and amygdala-seeded connectivity served as the outcome. This model also revealed a significant indirect effect (estimate = −1.18, 95% CI [−2.50, −0.07], $p = .033$); however, the direct effect also remained significant (estimate = −2.18, 95% CI [−3.65, −0.88], $p < .001$), with approximately 35.2% of the intervention's effect on amygdala connectivity explained by its effect on BOLD activation. That is, whereas most of the intervention's effect on BOLD activity was explained by its effect on amygdala-seeded connectivity, the opposite was not true, suggesting that the hypothesized mediation model (ie, amygdala connectivity as mediator and BOLD activation as outcome) best accounts for the relations among these variables.

DISCUSSION

The current study provides preliminary evidence for the causal role of an intervention targeting early parenting quality on amygdala-PFC function in response to face stimuli. We leveraged data from an RCT testing the efficacy of an early parenting intervention (ABC) for parents of infants at risk for maltreatment. As hypothesized, children of parents who received ABC exhibited greater PFC activation in response to faces than children of parents who received the control intervention, DEF. This extends previous work demonstrating that children from the ABC group show greater emotion regulation skills than their DEF counterparts[28] as well as a larger literature linking PFC

TABLE 1 Significant Attachment and Biobehavioral Catch-up (ABC)[a] > Developmental Education for Families (DEF)[b] Group Differences in Blood Oxygen Level–Dependent Activation (Fearful + Neutral Faces)

Cluster	Cluster size (voxels)	Center of mass (mm)			Peak 1 *p*	Hemisphere	Regions
		x	y	z			
2	8,764	16.6	10.1	24.7	.980	Right	Frontal pole, insular cortex, left/right superior frontal gyrus, left/right middle frontal gyrus, pars triangularis, pars opercularis, left/right precentral gyrus, temporal pole, superior temporal gyrus (anterior), postcentral gyrus, frontal medial cortex, left/right juxtapositional lobule cortex, subcallosal cortex, left/right paracingulate gyrus, left/right anterior-posterior cingulate gyrus, frontal orbital cortex, frontal operculum cortex, central opercular cortex, planum polare, Heschl gyrus, caudate, left/right thalamus, left/right putamen, pallidum, hippocampus
1	110	−43.6	−30.9	44.8	.956	Left	Precentral gyrus, postcentral gyrus, superior parietal lobule, anterior-posterior supramarginal gyrus

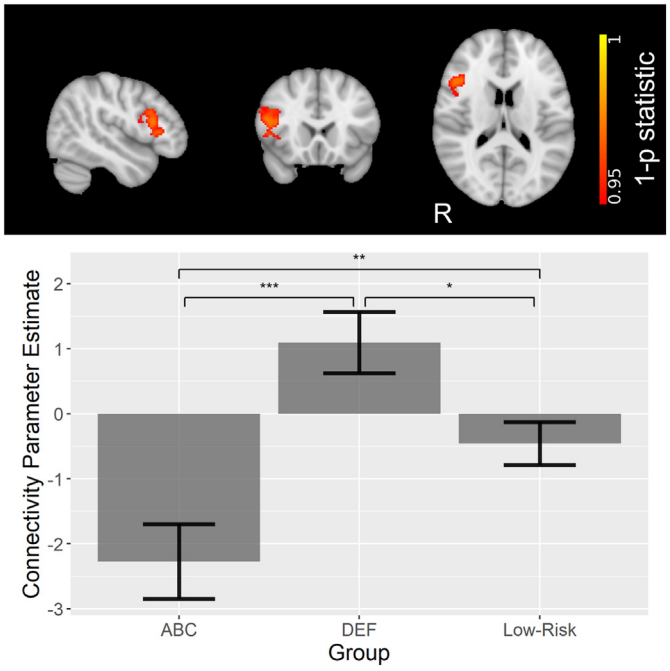
Note: Unless otherwise specified, regions listed correspond to the hemisphere(s) noted for the given cluster. Clusterwise p values < .05 adjusted for multiple comparisons. There were no significant clusters where DEF > ABC.
[a]*Experimental intervention.*
[b]*Control intervention.*

activation to emotion regulation strategies.[34] Supplementary analyses revealed that greater PFC reactivity to faces was associated with greater CBCL total problems scores, but only among the DEF group (Figure S7, available online), which also showed the least PFC reactivity to faces of the 3 groups. This pattern of findings may suggest that the low reactivity of the DEF group may be uniquely adaptive for this subset of children who experienced early adversity without intervention.

We also expected to see intervention effects on amygdala-PFC connectivity. Significant group differences did emerge. The ABC group showed negative connectivity between the amygdala and a cluster of brain regions including the right insula and right frontal orbital cortex, whereas the DEF group showed positive connectivity between the amygdala and these areas. Negative task-based connectivity indicates an inverse relation between the seed and the connected region; thus, in the ABC group, when PFC activity increased, amygdala activity decreased (and vice versa). The pattern of negative amygdala-PFC connectivity exhibited by the ABC group is common in adults, but children typically show positive or near-zero amygdala-PFC connectivity and gradually transition to more negative connectivity as they reach adulthood.[44] Because correlational studies demonstrate that children exposed to early adverse parenting show more negative amygdala-PFC connectivity than their nonexposed peers, it has been hypothesized that early life adversity may accelerate this shift.[12,19] Critically, however, the previous work was observational, leaving the cause of such precocious connectivity unclear.

Because we observed this precocious pattern only in at-risk children whose parents were randomly assigned to receive an intervention enhancing parental responsiveness and nurturance (ie, ABC), the present findings suggest that more negative amygdala-PFC connectivity in children is not caused by adverse parenting. Instead, highly sensitive parenting following early adversity could promote enhanced emotional development,[4] as possibly indicated by matured amygdala-PFC circuitry. This aligns with past work in at-risk children demonstrating improved cognitive flexibility,[27] decreased negative affect,[28] and improved autonomic regulation[31] following ABC relative to a control intervention—in some cases, many years after the intervention took place.[31] Of note, amygdala-PFC connectivity of both RCT groups significantly differed, in opposite directions, from that of the low-risk comparison group. The fact that participants in the DEF group showed more positive amygdala-PFC connectivity than their low-risk peers may suggest that early adversity in the absence of sensitive parenting may result in underdeveloped amygdala-PFC circuitry. Thus, sensitive parenting may have unique

FIGURE 2 Intervention Effect on Amygdala-Seeded Functional Connectivity to Faces



Note: *Colored regions indicate the significant cluster where control intervention (Developmental Education for Families [DEF]) > experimental intervention (Attachment and Biobehavioral Catch-up [ABC]) after correction for multiple comparisons. There were no significant clusters where ABC > DEF. Montreal Neurological Institute coordinates x = 48, y = 20, z = 16. Error bars indicate ± 1 SE. Low-risk group connectivity estimates are shown in bar graph for comparison. R = right. Please note color figures are available online.*
*p < .05; **p < .01; ***p < .001.

effects on children with a history of early life adversity compared with children without such histories. Internationally adopted children have significantly more economic and social resources than children in other adoptive and nonadoptive families, and these extra investments are associated with better educational outcomes.[45] These findings may help explain why more adultlike patterns of negative amygdala-PFC connectivity are observed in internationally adopted children who were previously institutionalized[12]; thus, future work examining the neurobiological consequences of early adversity might examine possible moderation by parenting quality. Another possibility is that the patterns of amygdala-PFC connectivity we observed may be partly explained by methodological differences across studies. That is, whereas the present study examined task-dependent BOLD connectivity across viewing of both fearful and neutral faces, past work in previously institutionalized children has focused on patterns of amygdala-PFC connectivity that significantly differed between facial expressions,[12] and previous animal work has examined rodent amygdala-PFC connectivity while at rest under light anesthesia.[11] Thus, heterogeneity of contrasts and scanning context may also help explain these disparate findings.

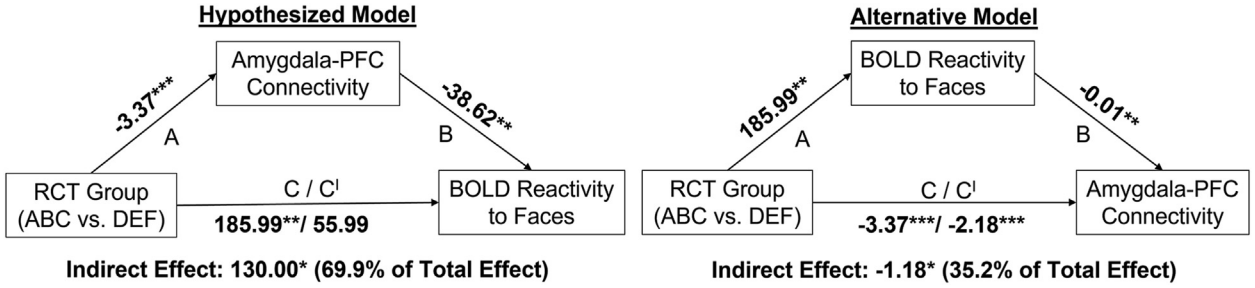
We also tested a hypothesis that a hierarchical relation between amygdala and PFC exists.[4,5] Consistent with this hypothesis, amygdala-PFC connectivity significantly mediated the relation between intervention group and children's neural responses to faces. Specifically, the effect of ABC on amygdala-PFC connectivity explained approximately 70% of the intervention's effect on BOLD responses to faces in large clusters of brain regions that included the anterior and posterior cingulate cortex, frontal orbital cortex, and other cortical and subcortical regions. A limitation of this mediation model was that both the mediator and the outcome variable were measured during the same fMRI assessment, limiting the ability to make firm claims about the sequence of effects. To establish temporal precedence of amygdala connectivity over brain responses to emotional stimuli more broadly, future work in this realm would benefit from

TABLE 2 Significant Developmental Education for Families (DEF)[a] > Attachment and Biobehavioral Catch-up (ABC)[b] Group Differences in Amygdala-Seeded Connectivity (Fearful + Neutral Faces)

Cluster	Cluster size (voxels)	Center of mass (mm)			Peak 1-*p*	Hemisphere	Regions
		x	y	z			
1	514	48.1	19.9	17.5	.973	Right	Frontal pole, insular cortex, middle frontal gyrus, pars triangularis, pars opercularis, precentral gyrus, postcentral gyrus, frontal orbital cortex, frontal operculum cortex, central opercular cortex

Note: *Unless otherwise specified, regions listed correspond to the hemisphere(s) noted for the given cluster. Clusterwise p value < .05 adjusted for multiple comparisons. There were no significant clusters where ABC > DEF.*
[a]*Control intervention*
[b]*Experimental intervention.*

FIGURE 3 Mediation Models for Intervention Effects on Amygdala-Seeded Functional Connectivity and Reactivity to Faces.



Note: *Intervention groups were coded as experimental intervention (Attachment and Biobehavioral Catch-up [ABC]) = 1, control intervention (Developmental Education for Families [DEF]) = 0; thus, positive estimates for group effects indicate greater scores in the ABC group than in the DEF group. The hypothesized mediation model revealed that 69.9% of the effect of ABC on blood oxygen level–dependent (BOLD) reactivity to faces was explained by amygdala-seeded connectivity. In contrast, the alternative model revealed that 35.2% of the effect of ABC on amygdala connectivity was explained by blood oxygen level–dependent activation, suggesting that the hypothesized model may better account for the relations among these 3 variables. PFC = prefrontal cortex.*

*$p < .05$; **$p < .01$; ***$p < .001$.

having earlier and repeated neuroimaging assessments. In the absence of additional neuroimaging time points, however, the present study also tested an alternative mediation model in which the mediator and outcome variables were switched. Together, the 2 models revealed that whereas most of the effect of ABC on BOLD responses to faces was explained by the effect of ABC on amygdala-PFC connectivity, the opposite was not true. This provides preliminary support for the idea that effects of early parenting quality may be mediated by amygdala connectivity.

In addition to the limitations mentioned above, it should be noted that the high-risk group likely included children with a range of adverse experiences. Because detailed CPS referral information was not available to research staff, we were unable to test for possible moderation of treatment effects by the specific type or severity of maltreatment a child experienced. However, even ostensibly distinct types of adversity (eg, abuse vs neglect) tend to co-occur, include overlapping kinds of experiences, and have shared biological and psychosocial consequences.[46] Furthermore, children with substantiated and unsubstantiated allegations of maltreatment experience similarly heightened risk for negative behavioral and developmental outcomes.[47] Together, this suggests it is unlikely that the specific type of adversity the child experienced would meaningfully moderate group effects. A second limitation concerns the interpretation of stimulus contrasts. Intervention effects were not evident in a fearful minus neutral face contrast and emerged only when combining the 2 facial expressions. This may be explained by the fact that children, especially children who have experienced early adversity, tend to perceive neutral facial expressions as more negative than older adolescents[48,49]; thus, both fearful and neutral faces may have been perceived as threatening. Without a third facial expression (eg, happy) or a nonface visual stimulus to act as a control, the present findings cannot rule out the possibility that intervention group differences were driven by an intervention effect on general visual processing, an intervention effect specific to processing faces, or an effect even more specific to threatening faces. To address this, future work in this vein may benefit from including a wider variety of visual stimuli. Third, participants from all 3 groups were predominantly African American. Although this may be considered a strength of the present study—as historical inequities in research practices have led to underrepresentation of Black participants in neuroscience research[50]—the racial/ethnic demographics of the present sample are not necessarily representative of the general population, therefore potentially limiting the generalizability of findings. Lastly, it should be noted that the lack of a preintervention fMRI assessment, coupled with the fact that not all randomly assigned participants were included in final analyses (eg, due to not participating in the fMRI substudy or due to excessive motion in the scanner), weakens the ability to draw firm causal conclusions based on the current imaging data. However, the fact that the numbers of attrited participants were similar across intervention groups may suggest that these attritional factors affected both groups in similar ways. Still, the final RCT sample of 41 participants was relatively small and raises the need to replicate the current findings in other, larger samples.

Nevertheless, the present study is the first to our knowledge to provide preliminary causal evidence in humans for the effect of early adverse parenting on amygdala-PFC connectivity and on PFC responses more broadly. Findings highlight amygdala-PFC connectivity as a potential key mediator of the effects of early parenting intervention on emotion regulation development in children. Results suggest that more negative amygdala-PFC connectivity observed among maltreated children may not

be caused by adverse parenting; rather, it may be that positive parent–child interactions following early adversity promote enhanced emotional development as indicated by matured amygdala-PFC circuitry. Findings further highlight the importance of considering scanning context (eg, task vs resting state) when interpreting the functional connectivity consequences of early adversity.

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