Discrimination of amygdala response predicts future separation anxiety in youth with early deprivation

Shulamite A. Green, 1 Bonnie Goff, 2 Dylan G. Gee, 3 Laurel Gabard-Durnam, 4 Jessica Flannery, 5 Eva H. Telzer, 6 Kathryn L. Humphreys, 2 Jennifer Louie, 2 and Nim Tottenham 4

1 Department of Psychiatry and Biobehavioral Sciences, University of California Los Angeles, Los Angeles, CA; 2 Department of Psychology, University of California Los Angeles, Los Angeles, CA; 3 Weill Cornell Medical College, New York, NY; 4 Department of Psychology, Columbia University, New York, NY; 5 Department of Psychology, University of Oregon, Eugene, OR; 6 Psychology Department and Beckman Institute for Science and Technology, University of Illinois, Champaign, IL, USA

Background: Significant disruption in caregiving is associated with increased internalizing symptoms, most notably heightened separation anxiety symptoms during childhood. It is also associated with altered functional development of the amygdala, a neurobiological correlate of anxious behavior. However, much less is known about how functional alterations of amygdala predict individual differences in anxiety. Here, we probed amygdala function following institutional caregiving using very subtle social-affective stimuli (trustworthy and untrustworthy faces), which typically result in large differences in amygdala signal, and change in separation anxiety behaviors over a 2-year period. We hypothesized that the degree of differentiation of amygdala signal to trustworthy versus untrustworthy face stimuli would predict separation anxiety symptoms. Methods: Seventy-four youths mean (SD) age = 9.7 years (2.64) with and without previous institutional care, who were all living in families at the time of testing, participated in an fMRI task designed to examine differential amygdala response to trustworthy versus untrustworthy faces. Parents reported on their children’s separation anxiety symptoms at the time of scan and again 2 years later. Results: Previous institutional care was associated with diminished amygdala signal differences and behavioral differences to the contrast of untrustworthy and trustworthy faces. Diminished differentiation of these stimuli types predicted more severe separation anxiety symptoms 2 years later. Older age at adoption was associated with diminished differentiation of amygdala responses. Conclusions: A history of institutional care is associated with reduced differential amygdala responses to social-affective cues of trustworthiness that are typically exhibited by comparison samples. Individual differences in the degree of amygdala differential responding to these cues predict the severity of separation anxiety symptoms over a 2-year period. These findings provide a biological mechanism to explain the associations between early caregiving adversity and individual differences in internalizing symptomology during development, thereby contributing to individualized predictions of future clinical outcomes. Keywords: Amygdala development; parents; stress; institutional rearing; separation anxiety; social.

Introduction

Unstable caregiving early in life, a significant source of adversity for the infant, has profound effects on emotional development. For example, extreme neglect during early institutional care significantly increases the risk for internalizing problems (Humphreys, Gleason et al., 2015; Slopen, McLaughlin, Fox, Zeanah, & Nelson, 2012; Zeanah et al., 2009). In childhood, these problems often manifest as separation anxiety symptoms (Cicchetti, Rogosch, & Toth, 1994; Wiik et al., 2011). Separation anxiety is a particular concern for youth with a history of institutional care (Elliott & McMahon, 2011; Humphreys, Lee et al., 2015; Tottenham et al., 2011), who lack a stable caregiver during infancy. At the neural level, this extreme form of neglect is associated with alterations in amygdala function (Gee, Humphreys et al., 2013; Maheu et al., 2010; Tottenham et al., 2011), a primary mediator of threat processes (Davis & Whalen, 2001). The amygdala, because of its rapid growth during the postnatal period (Gilmore et al., 2012) and its stress hormone receptor abundance (Avishai-Eliner, Yi, & Baram, 1996), is highly vulnerable to early adversity. These factors, combined with its associations with anxiety-related behaviors (Indovina, Robbins, Núñez-Eizalde, Dunn, & Bishop, 2011), suggest that the amygdala is a prime substrate for linking the well-characterized associations between caregiving adversity and heightened anxiety.

Despite exposure to adverse caregiving, there is nonetheless significant heterogeneity in internalizing outcomes, like separation anxiety, for previously institutionalized (PI) youth, which can be better understood by examining amygdala function. Cross-sectional studies have shown that individual differences in separation anxiety in PI youth are associated with amygdala circuitry (Gee, Gabard-Durnam et al., 2013). However, longitudinal designs permit for examination of the course of emotional difficulties, which have been shown to exacerbate with increasing age in PI youth (Castle et al., 2009; Colvert et al., 2008; Wiik et al., 2011), although in some cases the quality of the postadoptive home may buffer against some of these outcomes (Humphreys,
Gleason et al., 2015). The present study used a prospective design to assess whether amygdala function predicts increases in separation anxiety within PI youth.

Typically, the amygdala is highly sensitive to the safety/danger value of social-affective cues (Adolphs, 2010), and has been shown to be involved in the subtlest discriminations of facial cues, such as trustworthiness (Todorov & Engell, 2008), which both adults and children (Coggill & Banaji, 2015; Ewing, Caulfield, Read, & Rhodes, 2015) rapidly discriminate. However, for individuals with a history of adversity, this ability to reliably discriminate between cues of safety and danger can be compromised (reviewed in Christianson et al., 2012), and failure to exhibit discrimination either behaviorally or at the level of the amygdala has been associated with increased risk for internalizing psychopathology (Britton, Lissek, Grillon, Norcross, & Pine, 2011; Straube, Mentzel, & Miltner, 2005). Discrimination of the amygdala response to such cues may be predictive of anxiety outcomes for PI youth, as anxiety is associated with difficulty discriminating threat and safety cues (Lissek et al., 2009; Mohlman, Carmin, & Price, 2007), including very subtle discriminations based on trustworthiness (Meconi, Luria, & Sessa, 2014). In PI youth, amygdala function has typically been assessed by examining general reactivity to facial expressions (e.g. fear; Gee, Gabard-Durnam et al., 2013). Although this is a powerful probe of amygdala reactivity, these imaging studies have not tested discrimination ability. Indeed, other studies have shown that PI children have little difficulty behaviorally discriminating clear exemplars of facial expressions (Jeon, Moulson, Fox, Zeanah, & Nelson, 2010; Nelson, Parker, & Guthrie, 2006); however, signal detection methods have revealed a decreased sensitivity for subtler discriminations of facial differences in PI children (Fries & Pollak, 2004; Pollak, Cicchetti, Hornung, & Reed, 2000). These findings suggest that early deprivation might interfere with discriminating between subtle social-affective cues. Thus, the first aim of this study was to examine the ability of PI youth to discriminate between subtle social-affective cues (i.e. facial trustworthiness) both at the level of behavior and the amygdala.

We tested the hypothesis that the extent to which the amygdala exhibits a differential response to facial trustworthiness would predict age-related increases in separation anxiety. We examined separation anxiety symptoms because during childhood, internalizing problems commonly manifest as separation anxiety (Cartwright-Hatton, McNicol, & Doubleday, 2006) particularly in those who experience adverse caregiving (Cicchetti et al., 1994; Schimmenti & Bifulco, 2015) and because PI youth typically exhibit very high separation anxiety symptoms (Elliott & McMahon, 2011; Humphreys, Lee et al., 2015; Tottenham et al., 2011). Separation anxiety has been shown to be associated with amygdala alterations (Redlich et al., 2014) and weak regulatory connections between amygdala and prefrontal cortex (Carpenter et al., 2015; Gee, Gabard-Durnam et al., 2013), but the prospective role of amygdala function in child separation anxiety is still not well understood. Thus, we hypothesized that in contrast to youth with a typical caregiving history, on average PI youth would not exhibit differential amygdala responses to trustworthy and untrustworthy faces. However, the extent of differential amygdala response would predict the course of separation anxiety, informing a mechanistic understanding through which early caregiving adversity results in future elevations in separation anxiety.

Methods

Participants

Behavioral data (81 PI, 97 comparison) were collected from youth between 6 and 14 years old at Time 1, and 8 and 16 years old at Time 2 (see Figure 1) and functional MRI data were collected from a subset of these participants (39 PI, 66 comparison). Table 1 shows demographic data and group differences. PI youth had a history of institutional rearing and were adopted by families in the United States via international adoption (see online Appendix S1). Fifteen participants (4 PI; 11 comparison) were excluded due to amygdala parameter estimates more than 3 SD away the group mean, leaving a total fMRI sample of 33 PI and 41 comparison youth. Youth included in the behavioral study had reaction times of less than 1,000 msec and a false alarm rate of less than 50%, as well as reaction time data within 2 SD of the mean; the final sample included 42 PI and 45 comparison youth. Youth with a history of head trauma, seizure disorder, or IQ < 70 were excluded. All participants were right-handed. Families had incomes above the US median annual household income ($48,451; US Census Bureau, 2006). This study was approved by the UCLA

Figure 1 Ages of participants at Time 1 and Time 2. Participants with 1 point had data from the first scan but no follow-up information on anxiety.
Clinically elevated
RCADS separation
– Range 0

In the current study for examination of TrustA versus UntrustA
tural, happy, fear). The neutral faces were the stimuli of interest
identified as highly trustworthy (TrustA) or highly untrustwor-
neutral (target), happy, and fear faces]. However, face stimuli
performed a facial expression identification task [i.e. identify
€

During fMRI, participants completed two runs of a face-
processing task. Face stimuli were color images of four female
Faces were presented for 500 msec. The probability of a TrustA
index finger for each neutral face as quickly as they could.
fixed. Participants were instructed to press a button with their

Evaluation of anxiety (Time 1, Time 2)

Parents completed the Revised Children’s Anxiety and Depres-
sion Scale (Chorpita, Yim, Moffitt, Umemoto, & Francis, 2000)
Time 1 and again at Time 2 (2 years later). Parents rated 47
items describing their child’s mood and anxiety symptoms
(from ‘Never’ to ‘Always’). The separation anxiety scale was
used in this study, for example, ‘My child doesn’t like to be
away from his/her family,’ ‘My child gets scared if he/she
sleeps away from home.’ For ease of interpretation, T
scores and per cent clinically elevated (T score ≥ 65) are presented
in Table 1; raw scores were used for analyses. Parents rated their
own anxiety using the State

Stimuli

During fMRI, participants completed two runs of a face-
processing task. Face stimuli were color images of four female
faces (Lundqvist, Flykt, & Öhman, 1998). Overtly, subjects
performed a facial expression identification task [i.e. identify
neutral target expressions among a continuous sequence of
neutral (target), happy, and fear faces]. However, face stimuli
were selected from those models whose neutral faces have been
identified as highly trustworthy (TrustA) or highly untrustwor-
thy (UntrustA) by adult samples (Oosterhof & Todorov, 2008).
Thus, the stimulus set consisted of 12 unique stimuli (4
models [two TrustA and two UntrustA] x 3 expressions (neu-
tral, happy, fear). The neutral faces were the stimuli of interest
in the current study for examination of TrustA versus UntrustA
responses. Analyses did not include happy and fear as
emotional expressions can override trustworthiness qualities
of faces (Caulfield, Ewing, Bank, & Rhodes, 2015).

To ensure that children and adolescents could differentiate
the trustworthiness of the faces similar to adults, participants
rated the trustworthiness of the faces on a 1–9 Likert scale
(1 = not at all, 9 = extremely). For young participants, a
developmentally appropriate description of trustworthiness
was provided [e.g. friendly, nice, safe; Cogsdill & Banaji,
2015]. A repeated measures ANOVA showed a main effect of
face type [F(1,81) = 12.59, p = .001] such that all participants
rated TrustA faces as being more trustworthy than UntrustA
faces. There was no significant effect of Group or Age, or
Group x FaceType interaction.

fMRI task

We have reported on this task in previous publications [e.g.
Gee, Gabard-Durnam et al., 2013] with a focus on passive
viewing of emotion (i.e. fear), but the effect of face type (i.e.
neutral TrustA vs. neutral UntrustA) has not yet been exam-
ined. The task consisted of two counterbalanced runs: neutral
in the context of fear and neutral in the context of happy (with
an equal number of TrustA vs. UntrustA neutral faces in each
run). Stimulus order within each run was randomized and
fixed. Participants were instructed to press a button with their
index finger for each neutral face as quickly as they could.
Faces were presented for 500 msec. The probability of a TrustA
or UntrustA face was 50% on any given trial. Stimuli were
jittered (average 5,000 msec intertrial interval, 3,000–
9,000 msec) and randomized (Wager & Nichols, 2003). Each
run contained 48 trials (24 neutral target faces, 24 fearful or
happy nontarget faces). This task was administered both in
and out of the scanner as in-scanner behavior is not always a
reliable index of performance. Therefore, analyses focused on
the behavioral data from the out-of-scanner administration
and amygdala responses from the scanning session. The out-
of-scanner task was identical to the scanner task except that
neutral faces were presented in the context of happy nontarget
faces only, and stimulus duration was 500 msec with
1,000 msec intertrial intervals. Practice trials were adminis-
tered prior to both administrations of the task.

General procedure

Time 1 data collection involved two sessions. In the first
session, behavioral measures including RCADS and the out-of-
scanner face-processing task were collected. Participants were
acclimated to the scanner environment with an MRI replica. At
the second session [occurring within a mean of 3.6 months
(range = 0–16 months, SD = 3.6, 70% of subjects with data
from both sessions had the second session within 4 months of
the first], participants completed the fMRI face-processing
task. Time 2 collection of the RCADS occurred 2 years later
(mean = 23.6 months, SD = 3.4).

© 2016 Association for Child and Adolescent Mental Health.
fMRI data acquisition

Scanning was performed on a Siemens Trio 3.0 Tesla MRI scanner, with a 12-channel radiofrequency head coil. For each participant, an initial 2D spin-echo image (TR = 2,000 ms, TE = 40 ms, matrix size 256 × 256, 4 mm thick, 0 mm gap) in the oblique plane was acquired. A whole brain high-resolution, T1* weighted anatomical scan [MPRAGE; 256 × 256 in-plane resolution, 256 mm field of view (FOV); 192 mm × 1 mm sagittal slices] was acquired for registration and localization of functional data to Talairach space (Talairach & Tournoux, 1988). The faces task was presented on a computer screen through MR-compatible goggles during two functional scans. T2*-weighted echoplanar images were collected at an oblique angle of approximately 30° (130 volumes/run, TR = 2,000, TE = 30 ms, flip angle = 90°, matrix size 64 × 64, FOV = 192, 34 slices, 4 mm slice thickness, skip 0 mm, 24 observations per event type).

Behavioral data analysis

Accuracy was calculated as total correct presses to neutral faces minus total errors (commission to happy or fear faces and omission to neutral faces) for the two face types (TrustA, UntrustA) separately. We calculated mean reaction time for correct hits to TrustA and UntrustA neutral target faces and normed within-subject to account for age differences with the calculation (TrustA − UntrustA) / (TrustA + UntrustA). Accuracy and reaction time were calculated for the task both in and out of the scanner, and tested for interactions with age and FaceType (TrustA, UntrustA). IQ and sex were tested as potential covariates of the scanner, and tested for interactions with age and FaceType and reaction times were calculated for the task both in and out of the scanner, and tested for interactions with age and FaceType (TrustA, UntrustA). IQ and sex were tested as potential covariates but were nonsignificant, so were removed from final models.

In-scanner behavioral data. Reaction time and accuracy data collected in the scanner were used to ensure on-task performance. Overall, subjects showed good accuracy and there were no significant Group × FaceType interactions for accuracy (F = .59, p = .44; Mean for TrustA: CompAcc = 81%; PIAcc = 90%, and UntrustA: CompAcc = 75%; PIAcc = 76%) or reaction time (F = .17, p = .68; Mean (SD) for TrustA: CompRT = 682.15 (169.17) msec; PI RT = 694.07 (196.63) msec, and UntrustA: CompRT = 733.83 (205.93) msec; PI RT = 723.17 (179.0) msec).

Amygdala ROI. To examine group differences in amygdala responses, FaceType (TrustA, UntrustA) and hemisphere (left, right) were entered as within-subject factors in a repeated measures ANOVA, with group (PI, comparison) entered as a between-subject factor, and IQ as a covariate. Child sex was also tested as a covariate in this and subsequent models, but was nonsignificant in all analyses and thus removed from final models. There was a significant FaceType × Group interaction [F(1,71) = 4.04, p = .048] such that amygdala reactivity differentiated UntrustA versus TrustA faces for the comparison participants, but not for the PI participants (Figure 2B). This was due to the PI participants having increased amygdala response to the TrustA faces.

fmRI task

Whole brain analysis

To supplement the ROI analysis, a whole brain analysis was conducted to examine FaceType × Group differences. At the group level, a linear mixed effect whole brain analysis was conducted with within-subjects factors of face type and between-subjects factor of group, with age and IQ as covariates. This analysis was corrected for multiple comparisons at p < .01, using 3dClustSim, with a voxel size of 3 mm³, two-sided, third nearest neighbor, and a blur estimate (fwhm = 6).

Results

Behavioral task performance (out of scanner)

Two separate repeated measures ANOVA were performed with the within-subjects factor of FaceType (TrustA, UntrustA) and the between-subjects factor of group (PI, comparison) on the dependent measures of normed reaction time and accuracy. Age was entered as a covariate. There was a main effect of FaceType [F(1,87) = 5.88, p = .017], and a significant FaceType × Group interaction [F(1,87) = 4.02, p = .048]. Comparison participants had differential reaction times for the UntrustA versus TrustA faces, but the PI participants did not, due to slower reaction times to the TrustA faces (Figure 2A). There were no other main effects or interactions other than a Group × Age interaction indicating that for the comparison group, accuracy increased with age [F(1,81) = 8.48, p = .005]. Accuracy was not significantly correlated with reaction time in either group.
There were no other significant main effects or interactions.

**Whole brain analysis.** AFNI's 3dttest++ revealed significant FaceType × Group interactions in the left fusiform gyrus and right inferior parietal lobule (see online supplemental Table S2 and Figure S1). Post hoc examination showed that the PI group showed greater differentiation of UntrustA and TrustA faces, although the strength of these differentiations was not significantly associated with reaction time, adoption age, or trust ratings (ps > .05).

**Age of adoption and amygdala response**

To examine age of adoption associations, an UntrustA–TrustA difference score (hereafter referred to as DifferenceA) for beta weights was calculated so that a greater score indicated greater discrimination. Three youth placed in institutions after 24 months were excluded from this analysis because for this analysis, age of institutionalization serves as a proxy of ‘dose’ of institutional care, and children placed at later ages were outliers placed much later than other participants. A partial correlation (controlling for age) showed that left amygdala DifferenceA was significantly correlated with age of adoption \[ r(27) = .76, p < .001 \], with youth adopted at early ages having greater DifferenceA (Figure 3). There was a similar trend for the right amygdala \[ r(27) = .35, p = .06 \]. There was no significant correlation between DifferenceA and time with adopted family [left amygdala: \( r(30) = .24, p = .21 \); right amygdala: \( r(30) = .10, p = .61 \)], or between age of adoption and reaction time difference scores \[ r(35) = .10, p = .53 \].

**Prediction of Time 2 separation anxiety**

To test prospective associations between amygdala response and separation anxiety (SAD), a hierarchical regression was performed with Time 2 SAD score as the dependent variable and with Time 1 SAD score, child age at Time 1, IQ, Group (PI or comparison), DifferenceA, and a Group × DifferenceA interaction term entered as predictors (see Table 2) for those participants with follow-up data (19 PI, 20 comparison). Parent anxiety was initially tested as a covariate but was insignificant so removed from the final model. There were no significant differences in Time 1 SAD for participants who did versus did not return for follow-up. Right and left DifferenceA scores were highly significantly correlated with age of adoption [left amygdala: \( r(27) = .41, p < .001 \); right amygdala: \( r(30) = .40, p < .001 \)]. There was a trend in the right amygdala \[ r(30) = .32, p = .07 \], and there was no significant correlation between Time 1 SAD score and reaction time difference scores \[ r(35) = .10, p = .53 \].

![Figure 2](image1.png)

**Figure 2 Group differences in reaction time (A) and amygdala response (B) to UntrustA versus TrustA faces**

![Figure 3](image2.png)

**Figure 3 Association between age of adoption and amygdala difference score (UntrustA versus TrustA faces)**

<table>
<thead>
<tr>
<th>(a) Left amygdala</th>
<th>(b) Right amygdala</th>
</tr>
</thead>
<tbody>
<tr>
<td>( b^* )</td>
<td>( R^2 )</td>
</tr>
<tr>
<td>Step 1: SAD Time 1</td>
<td>.37*</td>
</tr>
<tr>
<td>Step 2: Demographics</td>
<td>.04</td>
</tr>
<tr>
<td>Time 1 age</td>
<td>-.05</td>
</tr>
<tr>
<td>IQ</td>
<td>-.23</td>
</tr>
<tr>
<td>Step 3: Group</td>
<td>.37*</td>
</tr>
<tr>
<td>Step 4: DifferenceA</td>
<td>.25*</td>
</tr>
<tr>
<td>Step 5: Group × DifferenceA</td>
<td>-.32*</td>
</tr>
</tbody>
</table>

*Standardized Betas in the final model.

\( p < .10; *p < .05; **p < .01; ***p < .001 \).
correlated in both the comparison and PI groups ($r = .92$ and .73, respectively, $p < .001$). To prevent collinearity problems, only right Difference$_A$ was entered into the regression (results from a separate regression including the left amygdala are provided in Table 2). Time 1 SAD significantly predicted Time 2 SAD. There was a Group x Difference$_A$ interaction indicating that in the PI group only, differential amygdala responses predicted Time 2 SAD over and above Time 1 SAD, such that youth with less discrimination between Untrust$_A$–Trust$_A$ had greater increases over the 2 years in SAD (Figure 4A). Functionally defined ROIs from the whole brain analysis (fusiform gyrus and inferior parietal lobule) did not significantly predict SAD.

A similar regression was conducted with normed reaction time difference score ($\text{Difference}_{\text{RT}}$) predicting Time 2 SAD after controlling for Time 1 SAD, age, and IQ ($n = 17$ PI; 19 comparison). In both groups, youth with lower Difference$_{\text{RT}}$ (indicating more similar reaction times to each face type) had greater increases in SAD (see Figure 4B and Table 3). The Group x Difference$_{\text{RT}}$ interaction term was not significant.

**Discussion**

This study examined differential amygdala responses to social-affective cues (i.e. trustworthiness) as a predictor of future separation anxiety in PI youth. We focused on amygdala function because of its demonstrated atypical development in PI populations (e.g. Tottenham et al., 2011), its role in discriminating trustworthiness (Todorov & Engell, 2008), and in separation anxiety (Redlich et al., 2014). While comparison youth exhibited differential amygdala responses to untrustworthy versus trustworthy faces (similar to adult samples (Oosterhof & Todorov, 2008), on average PI youth exhibited similar amygdala responses to both face types. This lack of discrimination was associated with older age at adoption. Behavioral data were consistent, showing that PI youth did not differentiate in reaction times. This lack of differentiation was striking given that both groups verbally indicated a difference in trustworthiness. Thus, while PI youth are able explicitly identify social-affective cues, they may be less likely to discriminate in terms of their rapid behavioral and amygdala responses (e.g. Olsavsky et al., 2013). Taken together, these findings suggest that the

![Figure 4](https://example.com/figure4.png)
Table 3: Regression predicting change in separation anxiety from reaction time discrimination (Difference\textsubscript{RT}, Untrust\textsubscript{A}–Trust\textsubscript{A})

<table>
<thead>
<tr>
<th>Step</th>
<th>Predictor</th>
<th>β*</th>
<th>ΔR\textsuperscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1: SAD Time 1</td>
<td>.44**</td>
<td>.29**</td>
<td></td>
</tr>
<tr>
<td>Step 2: Demographics</td>
<td>Time 1 age</td>
<td>−.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IQ</td>
<td>−.02</td>
<td></td>
</tr>
<tr>
<td>Step 4: Group</td>
<td>.001</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>Step 3: Difference\textsubscript{RT}</td>
<td>−.44*</td>
<td>.22***</td>
<td></td>
</tr>
<tr>
<td>Step 5: Group × Difference\textsubscript{RT}</td>
<td>−.09</td>
<td>.003</td>
<td></td>
</tr>
</tbody>
</table>

*Standardized Betas in the final model.

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

difficulty determining safety. It is also important to consider the potentially adaptive nature of indiscriminate social appraisals in PI youth (Chisholm, 1998). Future work may show that this framework is useful for understanding other atypical caregiver relationship patterns in PI youth beyond separation anxiety, including indiscriminate friendliness and/or disorganized attachment (e.g. Bos et al., 2011; Gleason et al., 2014).

A whole brain analysis showed that the PI group had greater differential responses in higher level face-processing cortical areas, unlike in the amygdala, possibly reflecting a greater reliance on top-down face-processing to differentiate the faces explicitly. Notably, these brain areas did not relate to age of adoption, anxiety, reaction time, or verbal trustworthiness ratings, suggesting that amygdala function is more directly linked with early adversity and likely to be a better predictor of future separation anxiety.

Study limitations include lack of information on specific preadoption adverse events (e.g. prenatal factors, multiple placements). However, the associations with age of adoption suggest that atypical discrimination is influenced by institutional care. The PI group differs on many levels from the comparison group, thus future studies should compare institutionalization with other forms of adversity. Additionally, the trustworthiness judgments in this study were implicit; possibly, PI children were less ‘tuned in’ to the trustworthiness aspect of the faces, perhaps because the overt task demanded more cognitive resources. However, this still suggests that PI youth have difficulty with implicit social-affective judgments, which is likely to affect their mental health outcomes (as shown here by their increases in separation anxiety). While the PI group showed less differentiated reaction time out of the scanner, both groups had similar differentiations of in-scanner reaction time. It is difficult to generalize in-scanner behavior as the scanner environment is highly novel, but further research is needed to understand reaction time differences in these two environments. Laterality of effects might have been underpowered (i.e. most effects showed similar trends in both hemispheres), and therefore conclusions about laterality should be tempered until further research is performed. Finally, our measure of separation anxiety is based on parent report, and should be considered a continuous measure of anxiety rather than a diagnostic measure.

Taken together, results of this study demonstrate that PI youth show reduced affective discrimination, both on a behavioral and neural level. Reduced discrimination was related to later age of adoption and also increases in separation anxiety over time. At the same time, these results are consistent with previous studies in suggesting that some children, especially those adopted at earlier ages, show resiliency in internalizing problems. These results
suggest a possible mechanism for increases in mental health difficulties often seen in youth with histories of early adversity, as well as a marker and predictor of variability and potential resiliency. While functional imaging is unlikely to be used for diagnostic purposes in the near future, these data can inform individualized predictors of the development of anxiety (e.g. lack of affective discrimination, social difficulties), which can be used to target early intervention and prevention of future clinical problems.

Supporting information
Additional Supporting Information may be found in the online version of this article:
Appendix S1. Supplementary methods.
Table S1. Country of origin for PI group.
Table S2. MNI coordinates for areas of significant between-group differences in response to UntrustA versus TrustA faces (FaceType × Group interaction).

Figure S1. Significant clusters for whole brain FaceType × Group interaction. Blue indicates regions where the PI group had greater UntrustA versus TrustA discrimination (PI > Comp; UntrustA > TrustA).

Acknowledgements
This work was supported by R01MH091864 (National Institute of Mental Health), the Dana Foundation, and Postdoctoral NRSA 1 F32MH105167-01. The authors report no biomedical financial interests or potential conflicts of interest.

Correspondence
Shulamite A. Green, Ahmanson-Lovelace Brain Mapping Center, University of California Los Angeles, 660 Charles E. Young Drive South, Los Angeles, CA 90095, USA; Email: shulamite@ucla.edu

Key points
- Early caregiving adversity is associated with increased anxiety symptoms and atypical amygdala function, which contributes to differentiating threat from safety in social-affective cues.
- Although caregiving adversity increases the risk for separation anxiety, many children show resilience, but the mechanisms of these individual differences are not well understood.
- This study examined amygdala function by focusing on discrimination of social-affective cues and tested the hypothesis that the degree to which the amygdala displays a typical differential response to social-affective cues will predict future increases in separation anxiety for previously institutionalized (PI) youth.
- For PI youth, the extent of differential amygdala response to social-affective cues longitudinally predicted future separation anxiety symptoms (across 2 years). Degree of amygdala differential response correlated with age of adoption.
- These results suggest a possible mechanism for increases in internalizing problems often seen in youth with histories of early adversity. We discuss these results in terms of individualized early intervention and prevention for this group of youth at risk for internalizing problems.

References

© 2016 Association for Child and Adolescent Mental Health.
Amygdala discrimination and anxiety after early deprivation


© 2016 Association for Child and Adolescent Mental Health.


Accepted for publication: 18 April 2016
First published online: 4 June 2016