Altered Amygdala Connectivity in Urban Youth Exposed to Trauma

Running head: Trauma disrupts amygdala connectivity

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Abstract:

Early life trauma exposure represents a potent risk factor for the development of mental illnesses such as anxiety, depression, and posttraumatic stress disorder. Moreover, deleterious consequences of trauma are exacerbated in youth living in impoverished, urban environments. *A priori* probability maps were used to examine resting-state functional connectivity of the amygdala in 21 trauma-exposed, and 21 age- and sex-matched urban children and adolescents (youth) without histories of trauma. Intrinsic functional connectivity analyses focused on amygdala-medial prefrontal circuitry, a key emotion regulatory pathway in the brain. We discovered reduced negative amygdala-subgenual cingulate connectivity in trauma-exposed youth. Differences between groups were also identified in anterior insula and dorsal anterior cingulate to amygdala connectivity. Overall, results suggest a model in which urban-dwelling trauma-exposed youth lack negative prefrontal to amygdala connectivity that may be critical for regulation of emotional responses. Functional changes in amygdala circuitry might reflect the biological embedding of stress reactivity in early life and mediate enhanced vulnerability to stress-related psychopathology.
Keywords

adolescent, child, maltreatment, resting-state, urban
1. Introduction

Estimates regarding rates of exposure to traumatic events in childhood range widely from 15-60% (Kessler et al., 1995, Dube et al., 2001, Stein et al., 2010), with strong evidence for the highest incidence occurring in urban, inner city settings (almost 90%; Gillespie et al., 2009, Goldmann et al., 2011). Trauma and stress injure the brain, precipitate cognitive-behavioral, emotional, and somatic problems, and are strong predictors of psychiatric illness (McEwen, 2012). Trauma in early life is especially harmful – associated with about 50% of childhood psychiatric disorders, and 30% of later-onset clinical disorders (Green et al., 2010).

Neurological evaluation of the link between trauma and psychiatric illness converges on amygdala and prefrontal brain regions. The amygdala is essential for the detection of threat and enhancement of vigilance (Zald, 2003), and is a central activator of the physiologic stress response (Dedovic et al., 2009). In contrast, the prefrontal cortex (PFC) is critical for regulation of emotion (Quirk and Beer, 2006). Animals that experience early life adversity show structural and functional changes in both the amygdala (see Malter Cohen et al., 2013b for a review) and PFC (review by McEwen and Morrison, 2013). Furthermore, early life adversity may also perturb the direct bidirectional connections between these two regions (review by Tottenham and Sheridan, 2009).

Research in humans and animals consistently demonstrates altered frontoamygdala connectivity is another consequence of early life stress (Gee et al., 2013a, Malter Cohen et al., 2013a, Philip et al., 2013, Grant et al., 2014). Compromised connectivity between the amygdala and PFC has been implicated in the pathophysiology of stress-related disorders. Specifically, altered frontoamygdala connectivity has been observed in anxiety (Kim and Whalen, 2009, Roy et al., 2013), depression (Tang et al., 2013), and posttraumatic stress disorder (PTSD; Edwards et al., 2013, Stevens et al., 2013, Brown et al., 2014). It is possible that altered frontoamygdala connectivity may emerge early in life, proximal to
negative traumatic experiences, and that this shift may be formative in determining healthy or deleterious outcomes for the individual. After all, frontoamygdala circuitry undergoes rapid changes across childhood and adolescence (Hare et al., 2008, Gee et al., 2013b, Gabard-Durnam et al., 2014), and thus alterations occurring during this period may have lasting effects.

While animal research has addressed embedding of stress exposure in frontoamygdala pathways in early life, research in humans is relatively recent. Gee (2013a) and Nooner (2013) provided the first studies of early life stress and functional connectivity (FC) in children/adolescents. Gee and colleagues examined task-related variation in FC associated with orphanage rearing in 6 to 17 year olds during a face-processing task, whereas Nooner and colleagues examined how trauma-symptoms in a healthy sample of community youth relate to variations in intrinsic FC during resting-state. Both studies evidenced altered frontoamygdala FC, with orphanage-reared children showing more medial frontal and typically developing youth showing more lateral frontal amygdala FC effects. Frontoamygdala FC alterations have also been shown to relate to trauma, diurnal cortisol, and internalizing symptoms in a longitudinal community sample of young adults (18 year olds; Burghy et al., 2012, Herringa et al., 2013). These studies compel the need for more research during formative years, prior to when psychopathology becomes chronic. Research that examines youth at high risk for developing clinical disorders could lead to identification of latent neural risk factors contributing to psychopathology in the aftermath of trauma exposure.

Here, we examine intrinsic resting-state neural FC in urban, low-income, minority trauma-exposed and comparison youth (ages 9-15). Past research shows not only that trauma frequency is extreme in African Americans living in impoverished urban areas, but also that negative consequences of trauma in urban, African American communities may be more severe (Alim et al., 2006). For example, while approximately 20% of trauma-exposed individuals in the general population subsequently develop
PTSD, African American urban residents who experience trauma are nearly two times more likely
develop PTSD (Goldmann et al., 2011). In addition, lower income is a significant predictor of more
severe emotional psychopathology following trauma (Lowe et al., 2014). Thus, additive effects of
trauma frequency and stress burden may be particularly deleterious to healthy emotional development.
Investigating the correlates of trauma exposure on the developing brain in a low income, African
American urban cohort of youth provides an opportunity to identify neurological changes in those who
are at highest risk for developing psychopathology.

2. Methods and Materials

2.1 Participants

The current study evaluated 42 urban youth, ages 9-15 (mean = 12.6, SD = 2.1). Participant ages
were selected to align with the emergence of puberty; puberty has been identified as a time when
affective disorders frequently manifest (Angold et al., 1998). The majority of participants (n = 27)
reported annual incomes of less than $40,000, and only a small number (n = 6) reported incomes greater
than $60,000. Participants were drawn from a larger study, and chosen to represent trauma-exposed (n = 21)
and comparison (n = 21) groups matched on age and sex. Data from 18 participants have been
reported previously (Thomason et al., 2013). Participants were recruited through advertisements posted
on the Wayne State University website, Craigslist (Detroit), printed flyers, or through Metro Detroit
mental health clinics. Exclusionary criteria included: English as a second language, lower than a 2nd
grade reading level, history of brain injury, neurological or movement disorders, or presence of
magnetic resonance imaging (MRI) contraindication. Parental informed written consent and
child/adolescent assent were obtained prior to participation. Demographic and behavioral measures were
administered during a laboratory visit, and MR imaging was performed during a subsequent visit (~2
weeks following the laboratory visit). All experimental procedures were approved by the Human Investigation Committee of Wayne State University.

### 2.2 Demographic data analysis

Highest level of parent/caregiver educational attainment and annual household income were coded as ordinal variables and compared between groups using Mann-Whitney U tests. Independent samples t-tests or chi-square tests were used to test for group differences in age, sex, IQ (derived from the Kaufman Brief Intelligence Test, version 2; Kaufman and Kaufman, 2004), parent report of child race/ethnicity, and pubertal development (using Tanner staging; Marshall and Tanner, 1968). Effects were considered significant at $p \leq 0.05$. Statistical analyses were 2-tailed, and implemented in IBM SPSS Statistics 21 (SPSS, Inc., Chicago, IL).

### 2.3 Trauma exposure

Utilizing both parent and child endorsements, participants that experienced at least one trauma indicated on the Children’s Trauma Assessment Center Screen Checklist were categorized as ‘trauma’ (source: Michigan Trauma Assessment Center). Forms of trauma included significant events that threatened their safety (e.g., physical or sexual abuse), undermined their security (e.g., neglect), caused unmanageable stress (e.g., witness a violent crime), or compromised interpersonal and family relationships (e.g., emotional abuse). Number and type of endorsed traumas are provided in Table I.

### 2.4 Self-reported affect measures

Participants completed two validated self-report questionnaires: the 10-item Children’s Depression Inventory (CDI; Saylor et al., 1984) and the 41-item Screen for Childhood Anxiety-Related Disorders (SCR; Birmaher et al., 1997). The SCR was administered during the lab visit and again at the MRI visit. Total SCR score was summed for each visit and then averaged, as lab and MRI scores were highly correlated, $r(42) = 0.76, p < 0.001$. A visual analog scale (VAS) was used to obtain an average rating of
fear/anxiety during the MRI visit (repeat measures at 30-minute intervals) as previously described (Thomason et al., 2013).

2.5 Imaging data acquisition

MRI data were acquired using a 3.0 Tesla Siemens Verio scanner. Participants were positioned in a 12-channel transmit-receive head coil and stabilized by padding to reduce motion-related artifacts. Participants were asked to lie quietly in the scanner with their eyes closed for the duration of the six-minute resting-state scan. For functional magnetic resonance imaging (fMRI), a total of 180 T2*-weighted blood oxygenation level-dependent (BOLD) images were acquired (interleaved ascending acquisition) using echo-planar imaging (EPI). The acquisition parameters were: repetition time [TR] = 2000 ms; echo time [TE] = 25 ms; flip angle = 90°; voxel size = 3.44 x 3.44 x 4 mm; matrix = 220 x 220; and 29 slices. Additionally, T1-weighted images were obtained for anatomical reference with the following parameters: time [TR] = 1680 ms; echo time [TE] = 3.51 ms; flip angle = 9°; voxel size = 0.7 x 0.7 x 1.3 mm; matrix = 256 x 256; and 176 slices.

2.6 Image preprocessing

Image preprocessing steps were conducted using SPM8 software (Statistical Parametric Mapping; http://www.fil.ion.ucl.ac.uk/spm/). After discarding the first 4 EPI volumes to allow for signal stabilization, images were slice-time corrected, realigned, spatially normalized to the Montreal Neurological Institute (MNI) template, and smoothed using an 8-mm Gaussian kernel. Frame-to-frame excursion, root mean square (rms), and head movement across the scan were calculated and averaged for translational (x, y, z) and rotational (roll, pitch, yaw) movement directions. Motion parameters were compared between groups using 2-tailed independent samples t-test. Between group motion differences were considered significant at \( p < 0.05 \).

2.7 Seed-based connectivity analysis
Given prior research showing altered intrinsic FC of specific amygdala subregions in adults with PTSD (Brown et al., 2014) and anxiety (Etkin et al., 2009), connectivity of centromedial (CM) and basolateral (BL) amygdala were evaluated separately. Amygdala subregions were the leading choice for seeded ROI analyses because of their unique connectivity profiles. For more detailed discussion of amygdala subregion connectivity in humans, see (Roy et al., 2009). The present study was centered on CM and BL subregions as these have previously been implicated in stress-related psychiatric disorders (Etkin et al., 2009, Brown et al., 2014). Seed regions were defined for CM and BL amygdala structural subdivisions (Amunts et al., 2005), following prior work (Roy et al., 2009, Qin et al., 2012). In brief, bilateral masks used stereotaxic, probabilistic maps of cytoarchitectonic boundaries defined by the SPM Anatomy toolbox (Eickhoff et al., 2005). Cytoarchitectonic maps show high reliability and accuracy for guiding anatomical segmentation of amygdala subregions in children as young as six years of age (Kim et al., 2010, Qin et al., 2012). Given that we had no a priori lateralization hypotheses, we averaged signal from right and left amygdala masks. FC of amygdala subregions was determined by semipartial correlation using the CONN Functional Connectivity Toolbox (ver.12.p; www.nitrc.org/projects/conn). Between group effects were considered within an anatomically defined medial prefrontal region used in prior works (Etkin and Schatzberg, 2011, Marusak et al., 2014). This mask was selected to encompass perigenual (pgACC) and subgenual (sgACC) regions of the anterior cingulate, as these regions suppress limbic reactivity through direct connections to the amygdala. Family-wise error (FWE) corrected $p < 0.05$, significance level was used.

Motion poses a significant source of noise in FC analyses. None of the participants included in the present study had motion exceeding 1.5 mm in any direction. We addressed residual motion-related artifacts in three steps. First, Siemens MRI motion correction (MoCo) software was used during image acquisition. This procedure retrospectively measures 6 parameters of rigid-body translation and rotation,
and produces a corrected time series using affine transformation. Second, functional image volumes were realigned to the mean image in SPM8. Third, realignment parameters (with another six parameters representing their first order temporal derivatives) were removed with covariate regression analysis before computing amygdala FC. Signals from white matter and cerebral spinal fluid were also regressed out using anatomical component correction (aCompCor; Behzadi et al., 2007, Chai et al., 2012). Low overall movement levels and lack of differences between groups augment confidence that motion did not compromise observed effects.

Secondary whole-brain analyses were performed to comprehensively evaluate connectivity of the amygdala. In addition to CM and BL regions, a superficial (SF) amygdala subregion mask was generated using procedures described above, and connectivity from this area was also examined for possible differences between groups. Regions showing altered FC between groups were reported at a threshold of $p < 0.005$, cluster minimum = 10 voxels. This threshold was derived from suggested standards for whole-brain comparisons (Lieberman and Cunningham, 2009). Results that survived multiple comparisons correction for the whole brain (spatial cluster extent threshold; > 158 voxels for $p < 0.05$ FWE corrected) are denoted with an *asterisk in Supplementary Table 1. To plot the direction of trauma-related amygdala connectivity, individual participant beta values were extracted from peaks using 4 mm radius spheres. Pearson correlation was used to test for associations between amygdala FC and anxiety, depressive symptoms, IQ, and income. All coordinates are reported in Montreal Neurological Institute (MNI) convention.

To further validate our findings, data were re-analyzed using motion “scrubbing” (Power et al., 2012). Specifically, movement was plotted and visually inspected for each participant using ArtRepair software (http://cibsrr.stanford.edu/tools/human-brain-project/artrepair-software.html). Through this process we identified 4 trauma and 3 comparison participants that required censorship. They were
scrubbed, and following this, maximal movement for all individuals in the sample was reduced to 0.171 mm and 0.392 degrees. Major comparisons and study outcomes were evaluated using this alternative analytic scheme, and those data are reported as supplementary material.

3. Results

3.1 Participants

3.1.1 Demographics and movement. As shown in Table I, groups did not differ in age, pubertal maturation, sex, race/ethnicity, parental educational attainment, or movement during the resting-state scan. Overall, residual movement was well within accepted standards (< 1.5 mm translational rms; cf. Fair et al., 2012). Relative to comparison youth, trauma-exposed participants reported lower levels of household income and IQ (see Table I), effects that were anticipated based on prior work (e.g. De Bellis, 2001, Lantz et al., 2005). Follow-up analyses controlling for income and IQ were conducted on FC data, as described below. There were significantly more females than males represented across the entire sample, ($\chi^2_{(1)} = 6.1, p = 0.014$). Distribution of participant race/ethnicity across the sample was representative of the study community (Wayne Country, Michigan; $\chi^2_{(2)} = 4.09, p = 0.13$; www.census.gov). 4 participants (3 trauma, 1 comparison) were on psychotropic medications (1 on stimulants, 1 on selective serotonin reuptake inhibitors, 2 on serotonin-norepinephrine reuptake inhibitors, and 1 on beta-2 adrenergic agonists). Medications were not withheld for scanning. Follow-up analyses excluding participants on medications yielded no changes to observed effects.
3.1.2 Self-reported affect measures. Although the trauma group was not chosen on the basis of psychopathology, participants with histories of trauma reported higher levels of anxiety (SCR) relative to comparison participants (Table I). This is consistent with reports that childhood trauma exposure is a strong predictor of emotional psychopathology (Kessler et al., 1997). Follow-up analyses were

<table>
<thead>
<tr>
<th>Variable</th>
<th>Trauma (n = 21)</th>
<th>Comparison (n = 21)</th>
<th>Group Comparison (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, m (SD)</td>
<td>12.77 (2.00)</td>
<td>12.32 (2.19)</td>
<td>ns</td>
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<tr>
<td>Pubertal Maturation (Tanner Stage), m (SD)</td>
<td>3.75 (0.95)</td>
<td>3.12 (1.38)</td>
<td>ns</td>
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<tr>
<td>Sex (Female), n (%)</td>
<td>15 (71.43)</td>
<td>14 (66.67)</td>
<td>rs</td>
</tr>
<tr>
<td>IQ, m (SD)</td>
<td>90.55 (10.9)</td>
<td>109.94 (16.04)</td>
<td>&lt; 0.001</td>
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<tr>
<td>Race/Ethnicity, n (%)</td>
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<td></td>
</tr>
<tr>
<td>African American</td>
<td>10 (47.62)</td>
<td>10 (47.62)</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>4 (19.05)</td>
<td>10 (47.62)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
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<td>0 (0)</td>
<td></td>
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<tr>
<td>Not reported</td>
<td>4 (19.05)</td>
<td>1 (4.76)</td>
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</tr>
<tr>
<td>Annual Household income, n (%)</td>
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<td>Less than $40,000</td>
<td>17 (81)</td>
<td>10 (47.6)</td>
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<tr>
<td>$40,000 - $60,000</td>
<td>1 (4.8)</td>
<td>7 (33.3)</td>
<td></td>
</tr>
<tr>
<td>$60,000 - $80,000</td>
<td>2 (9.5)</td>
<td>1 (4.8)</td>
<td></td>
</tr>
<tr>
<td>$80,000 - $100,000</td>
<td>0</td>
<td>1 (4.8)</td>
<td></td>
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<tr>
<td>Over $100,000</td>
<td>0</td>
<td>2 (9.5)</td>
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</tr>
<tr>
<td>Not reported</td>
<td>1 (4.8)</td>
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<td>Highest Level of Parental Education, n (%)</td>
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<tr>
<td>No GED/ no high school diploma</td>
<td>2 (9.5)</td>
<td>1 (4.8)</td>
<td></td>
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<tr>
<td>GED/ high school diploma</td>
<td>4 (19)</td>
<td>1 (4.8)</td>
<td></td>
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<tr>
<td>2-year degree or some college</td>
<td>8 (38.1)</td>
<td>9 (42.9)</td>
<td></td>
</tr>
<tr>
<td>4-year degree</td>
<td>3 (14.3)</td>
<td>7 (33.3)</td>
<td></td>
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<tr>
<td>Masters</td>
<td>3 (14.3)</td>
<td>2 (9.5)</td>
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<tr>
<td>Doctorate</td>
<td>0</td>
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<tr>
<td>Not reported</td>
<td>1 (4.8)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Type of Trauma Endorsed*, n (%)</td>
<td></td>
<td></td>
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<tr>
<td>Physical abuse</td>
<td>4 (19%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Neglectful home environment</td>
<td>3 (14%)</td>
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</tr>
<tr>
<td>Emotional abuse</td>
<td>2 (10%)</td>
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<td></td>
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<tr>
<td>Exposure to domestic violence</td>
<td>14 (67%)</td>
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<td></td>
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<tr>
<td>Exposure to any other violence not already identified</td>
<td>11 (52%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Multiple separations from parent or caregiver</td>
<td>4 (19%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Sexual abuse or exposure</td>
<td>5 (24%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Anxiety Symptoms (SCR)*, m (SD)</td>
<td>22.97 (16.49)</td>
<td>14.23 (11.08)</td>
<td>0.05</td>
</tr>
<tr>
<td>Depressive Symptoms (CDI)*, m (SD)</td>
<td>4 (5.32)</td>
<td>1.8 (2)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Note: *Trauma criteria are from the Children’s Trauma Assessment (CTA) Center Screen Checklist (Item 1) by the Michigan Trauma Assessment Center. *SCR = Screen for Child Anxiety-Related Emotional Disorders. *CDI = Children’s Depression Inventory. All p-values derived from t-tests with the exception of sex and race/ethnicity comparisons, which used chi-square tests, and income and parental education, which used Mann-Whitney U tests. Abbreviations: m, mean; SD, standard deviation; n, number; ns, not significant; max, maximum; rms, root mean squared (head position change during the resting-state scan).
conducted to account for effects of anxiety on connectivity (see below). While anxiety scores reported during lab and MRI visits were significantly correlated within subject (see Methods), it is possible that variation across visits differed between groups. We therefore tested for effects of visit on anxiety levels using a Group (trauma, comparison) x Visit (lab, MRI) ANOVA. Consistent with group differences reported above, a significant main effect of Group emerged, $F(1, 80) = 7.08, p < 0.009$. No main effect of Visit, or Group x Visit interaction was observed ($p$'s > 0.4) suggesting that trait anxiety was stable over a period of two weeks and variability between visits did not differ between groups. In contrast, average state levels of fear/anxiety during the MRI visit (VAS) did not significantly differ between groups, $t(39) = 0.3, p = 0.77$. Given that VAS ratings were previously shown to correlate with cortisol reactivity during the scan (Thomason et al., 2013), this result suggests that connectivity differences are not likely influenced by group differences in biologic stress responsivity during the MRI visit. Groups did not differ on trait levels of depressive symptoms, $p > 0.08$ (see Table I).

3.2 Lack of amygdala-sgACC connectivity in trauma-exposed youth

We observed significant group differences in CM amygdala-sgACC connectivity ($x = 8, y = 18, z = -8, p_{FWE} = 0.022, Z = 3.80$). Extraction of average connectivity strength within this cluster revealed that the group effect resulted from the predicted negative amygdala-sgACC FC in comparison participants, which was absent in trauma-exposed youth (Figure 1). CM amygdala-sgACC FC was not related to anxiety (SCR, $r(42) = 0.01, p = 0.92$) or depressive (CDI, $r(42) = 0.03, p = 0.83$) symptoms across the sample, or within the trauma group (SCR, $r(21) = -0.22, p = 0.34$; CDI, $r(21) = -0.15, p = 0.51$). Group differences in CM amygdala-sgACC connectivity remained significant when controlling for IQ (peak at $x = -12, y = 40, z = -4, p_{FWE} = 0.025, Z = 3.77$) and income (peak at $x = 10, y = 22, z = -10, p_{FWE} = 0.027, Z = 3.74$). No significant effects of trauma were observed for BL amygdala-sgACC connectivity at $p < 0.05$ FWE-corrected.
**Figure 1. Absent negative centromedial (CM) amygdala-subgenual cingulate (sgACC) negative connectivity in trauma-exposed youth.** Left: Signal was averaged across anatomically-defined bilateral CM amygdala source region. Right: Tukey’s boxplots depict connectivity values by group centered on the sgACC peak. The middle line indicates the median, vertical line the range, and the limits of the box represent upper and lower quartiles. Results significant at a threshold of $p < 0.05$, FWE-corrected.

### 3.3 Divergent patterns of amygdala subregion FC across the sample

Secondary whole-brain analyses were performed to examine connectivity of major amygdala subregions. FC maps across the entire sample (Figure 2) revealed unique patterns of connectivity for bilateral CM, BL, and SF amygdala subregions that are consistent with previous cytoarchitectonically-based amygdala FC studies (e.g. Roy et al., 2009, Brown et al., 2014). Briefly, CM showed signal covariation with striatal regions, whereas BL showed signal covariation with temporal and frontal cortical regions. SF signal was strongly correlated with signal in limbic regions.
Figure 2. Functional connectivity of amygdala subregions across the entire youth sample. Left: Inset shows anatomically-defined bilateral amygdala seed regions: CM (centromedial; red), BL (basolateral; blue), and SF (superficial; green). Axial slices show brain areas positively correlated with amygdala seed regions. Right: Surface renderings are used to depict separation and overlap of amygdala neural networks. Connectivity maps displayed at $p < 0.005$, cluster minimum = 10 voxels.

3.4 Trauma effects on amygdala whole-brain connectivity

Extensive differences in amygdala FC were observed between groups (see Table S1). We also found that in a subset of areas in which groups differed, FC also related to income, IQ, anxiety, or depressive symptoms. Thus, not only does amygdala FC vary across brain areas, but this variation relates to symptom severity. Data are summarized in Figure 3 as radar plots. Visual inspection of these plots suggests that, overall, individuals who have experienced trauma demonstrate increased positive connectivity across widespread brain regions. Consistency was observed across amygdala subregions in that frontoamygdala connectivity was negative in the comparison group but not in trauma-exposed youth (see Figure 4). This was also true in anterior insula-amygdala connectivity, that was more negative in comparison but not in trauma-exposed participants for the SF subregion. A different effect was observed
for the dorsal anterior cingulate (dACC); there, we observed increased positive amygdala connectivity in comparison but not trauma participants (Figure 4).

Figure 3. Differential patterns of amgydala subregion functional connectivity (FC) across the whole brain in trauma-exposed (purple) and comparison (blue) youth. Numerical values represent the average within-group correlation strength in areas where groups differed, across frontal, limbic, parietal, subcortical, and temporal/occipital regions (coordinates are provided in Table S1). Wider area on radial plots in trauma-exposed youth suggests a pattern of enhanced positive FC of the amygdala to many brain regions, particularly to areas of the frontal cortex. Dashed line indicates zero; the middle of the plot indicates negative values. Abbreviated brain regions are defined in Table S1.
Figure 4. Regions showing group differences (trauma, purple; comparison, blue) in functional connectivity with BL (basolateral) and SF (superficial) amygdala. Right: Tukey’s boxplots depict connectivity values by group. The middle line indicates the median, vertical line the range, and the limits of the box represent upper and lower quartiles. Corresponding MNI coordinates of peak group difference (see Table S1) are indicated adjacent to each boxplot. Results are shown at $p < 0.005$, cluster minimum = 10 voxels, for display purposes. Abbreviations: SFG, superior frontal gyrus; OFC, orbitofrontal cortex; dACC, dorsal anterior cingulate; INS, insula.

3.5 Replication of results in scrubbed data

Data were reanalyzed utilizing censoring of high-movement frames. Following censoring, average group motion was $< 0.05$ mm and $< 0.002$ degrees rms. As shown in Figure S1, main findings of amygdala FC across the sample (Figure 2), and group differences in FC in the sgACC (Figure 1), superior frontal gyrus, dACC, and insula (Figure 4) were replicated. However, the group difference in BL amygdala-orbitofrontal cortex connectivity was no longer significant.

4. Discussion
The present study evaluates links between early life trauma and neural circuit organization, within a high sociodemographic risk youth sample. Our results indicate reduced negative amygdala-sgACC FC in trauma-exposed youth, fitting with prior observed associations between disrupted amygdala circuitry and emotional psychopathology. For example, alterations in frontoamygdala circuitry have been reported in adults with PTSD (Brown et al., 2014), and adolescents (Roy et al., 2013) and adults with generalized anxiety disorder (Etkin et al., 2009). We add to this conceptualization by discovering that experience (trauma) is associated with altered intrinsic amygdala FC in formative, developmental years. In particular, trauma-related changes were observed within amygdala-sgACC circuitry, a pathway that is critical for emotion regulation. These findings are in line with our recent study showing a reduced ability to regulate emotional conflict and an absence of negative frontoamygdala connectivity during conflict resolution in trauma-exposed youth (Marusak et al., 2014). The present findings extend this work by demonstrating that deficits in this critical emotion regulatory pathway are present even when individuals are not engaging regulatory control processes.

Four studies most similar to the current investigation are summarized in Supplementary Table 2. The most comparable study, implemented by Gee and colleagues, evaluated FC in orphanage-reared youth during a face processing task (Gee et al., 2013a). Focusing on the same frontoamygdala circuitry emphasized here, they too found abnormalities in neural FC between groups. Interestingly, their effects were related to the type of emotional face being processed. That is, between group differences were noted during the processing of fear faces, but the direction of effects differed during processing of happy faces. This result, paired with data presented here about intrinsic FC during resting-state suggests that dynamics in frontoamygdala circuits in trauma-exposed youth may relate to processing demands, and results obtained should be considered in that light. Gee and colleagues also provide evidence that the age of participants being examined may interact with observed effects. They found that comparison and
orphanage-reared participants exhibited different patterns of age-related change in frontoamygdala FC to fear faces. Specifically, between group differences appear to have been significant in children but not adolescents. This could be interpreted as a reduction in differences between groups with age. Though the cross-sectional nature of this sample precludes direct evaluation of this possibility, this observation can be regarded in consideration of current theory that principals of neural connectivity at one point in life may be adaptive, while at another may confer risk (Tottenham, 2013).

We interrogated amygdala FC from three amygdala subregions. CM amygdala is involved in allocating attention to relevant stimuli (Davis and Whalen, 2001) and mediating increased vigilance (Dringenberg and Vanderwolf, 1996). In contrast, BL amygdala has been linked to associative learning processes, and the SF amygdala has been implicated in social/affective processing (LeDoux, 2003, Phelps and LeDoux, 2005, Goossens et al., 2009). Given that amygdala subregions exhibit unique developmental patterns of FC across childhood and adolescence (Gabard-Durnam et al., 2014), it is likely that trauma impacts these circuits in different ways. We support this supposition by having attained commonalities and discrepancies in effects across subregions. Commonalities were observed across amygdala subregions such that youth that endured traumatic experiences tended to have diminished frontoamygdala connectivity. Discrepancies were noted for regions of the striatum, parietal regions, and regions comprising the default mode brain network (e.g., precuneus and posterior cingulate). For these areas, CM and BL showed trauma-related FC effects but SF did not. In contrast, analysis of SF connectivity revealed trauma effects in regions that comprise the salience network of the brain, the insula and dACC, whereas other subregions did not. Thus, amygdala subregions critical for learning and attention and for social/affective processing demonstrate overlapping, but also distinct trauma-related alterations in connectivity.
Our results support a model of reduced emotion regulatory control in urban youth with histories of trauma. Amygdala-medial prefrontal correlations are negative at rest (Gee et al., 2013b) and during the regulation of emotional responding (Hare et al., 2008), a pattern of connectivity thought to index top-down regulatory control. Behaviorally, loss of inhibitory affective control has been associated with exposure to trauma (Pechtel and Pizzagalli, 2011) as well as presence of clinical mood disorders (Etkin et al., 2013). Prior research has also described negative attention bias and augmented vigilance in those with histories of early life trauma exposure (Pollak, 2008). Furthermore, studies using task-based fMRI show hyperactivity of the amygdala and/or hypoactivity of medial prefrontal regions in individuals with anxiety (McClure et al., 2007), PTSD (Etkin and Wager, 2007), and in children with histories of early adversity (Tottenham et al., 2011, McCrory et al., 2013), which may reflect failure of prefrontal regions to regulate amygdala reactivity. The direction of our effects (more negative frontoamygdala FC in comparison participants) suggests reduced top-down control via frontoamygdala neurocircuitry may be a consequence of early traumatic experiences.

Whole-brain exploratory analyses identified stronger amygdala-anterior insula connectivity in trauma-exposed youth. Increased FC indicates greater signal covariance between regions that detect threat and generate fear responses (i.e., amygdala; LeDoux, 2003) and process meaning and prediction of aversive bodily states (i.e., insula; Craig, 2011). Stronger amygdala-insula FC has also been reported in adults with PTSD (Rabinak et al., 2011) and adolescents with GAD (Roy et al., 2013), highlighting the relevance of this pathway for clinical disorders. Moreover, task-based fMRI studies have demonstrated increased amygdala and insula reactivity in children exposed to violence (McCrory et al., 2011) and soldiers that endure combat stress (van Wingen et al., 2011). Increased functional covariance in the amygdala and insula at rest suggests they may be primed for rapid co-activation, or that functions subserved by these regions tend to be more coordinated.
Our results indicate significantly reduced (positive) amygdala-dACC connectivity in trauma-exposed youth. Whereas ventral aspects of the ACC (e.g., perigenual and subgenual regions) are implicated in emotion regulation, dACC is associated with the expression of fear (Etkin et al., 2011). Evidence suggests that top-down (i.e., dACC/lateral prefrontal-based) forms of emotion regulation work by recruiting the ventral ACC (vACC), which dampens limbic reactivity directly (Etkin et al., 2011). Fitting with this conceptualization, our data show congruent between group effects in dACC and sgACC. Dorsal aspects of the ACC are also implicated in sympathetic nervous arousal (Critchley, 2005). Thus, our altered amygdala-dACC FC results may reflect aberrant modulation of autonomic nervous system function. This is consistent with reports of increased arousal and fear in trauma-exposed youth, which may contribute to the development of clinical disorders (e.g., anxiety; Glaser, 2000).

We observed that differences in neuroconnectivity between trauma-exposed and control groups were associated with depression or anxiety symptomology only in select regions, including the sensorimotor cortex, middle temporal gyrus, and superior parietal cortex. This is highly consistent with the observation that trauma exposure does not precisely predict emergence of psychopathology. Considerable data has shown that outcomes resulting from trauma exposure vary greatly across individuals (Cicchetti and Rogosch, Felitti et al., 1998, Tottenham and Sheridan, 2009). Thus, one would not expect that the relationship between brain measures and previously experienced trauma would be ubiquitously significantly correlated. These variables are neither orthogonal, nor perfectly correlated. Thus, having observed some but not complete overlap in significance could have been predicted. Similar consideration could be predicted for observed associations in income and IQ, all of which are presented in superscript in Supplementary Table 1.

Given increased awareness that motion confounds interpretation of fMRI data and the resulting shift toward rigorous approaches to dealing with motion in resting state data, we reanalyzed all data using an
alternative scrubbing approach (Deen and Pelphrey, 2012, Van Dijk et al., 2012, Power et al., 2015). We saw that observed main effects were consistent, with the exception of a group difference in the orbitofrontal cortex that did not hold after scrubbing. It is possible this result did not replicate because the group difference covered a smaller territory to begin with (15 voxels), and because clusters smaller than 10 contiguous voxels are not reported as significant. The reason that in large part we saw consistent effects in scrubbed and non-scrubbed data may be due to the low overall movement profile of participants in the original analyses.

Our results underscore that traumatic stress may alter limbic circuitry in the immature brain and this may precede the onset of clinically significant conditions. There is evidence suggesting that changes in frontoamygdala circuits persist even decades later into adulthood (Dannlowski et al., 2012), emphasizing the relevance of early life exposures for lifelong socioemotional functioning. While altered amygdala connectivity may reflect ontogenetic adaptation to an unsafe rearing environment, it is possible that these neural changes confer elevated threat vigilance, which may be detrimental to healthy emotional development. For instance, it has been suggested that increased amygdala-insula coupling mediates anxious anticipation of negative events (Carlson et al., 2011). Recalibration of neural connectivity in limbic circuitry in early life may be detrimental to evaluation of threat and safety, and compromise a child’s ability to master age-appropriate skills in social and cognitive domains. For example, altered connectivity may reflect changes in synaptic function within this emotion regulatory network or reduced priming of network components that allow for rapid control of emotional responding.

Our findings should be considered in light of limitations. First, we were not sufficiently powered to differentiate results based on onset (age) or type of trauma. While retrospective analysis shows that trauma onset and trauma type relate to distinctive patterns of emotional functioning (English et al.,
prior studies also document nonspecific effects of trauma type on outcomes (Arata et al., 2007, Collishaw et al., 2007) and some suggest that disentangling unique effects may result in overly narrow interpretations (Green et al., 2010). Next, participants were drawn from a larger study, and were not selected on the basis of IQ or income. As a result, groups were not matched on these variables. This is not surprising given the strong association between these variables and trauma prevalence (De Bellis, 2001), but also not ideal for disentangling connectivity effects. However, the alternative is to select matched samples that may not convey the natural conditions present in trauma with regard to neural connectivity. Moreover, we are assured by the consistency of our findings with both animal and human research (Gee et al., 2013a, Malter Cohen et al., 2013a, Brown et al., 2014), and because follow-up analyses indicated that amygdala-sgACC results held when controlling for income and IQ. In addition, we provide areas that are influenced by trauma exposure and other risk factors (i.e., poverty, IQ, anxiety, depression). Interactions between these variables are areas for future research. While we did not find an association between frontoamygdala connectivity and anxiety or depressive symptoms, it is possible that additional symptom-brain associations would be detected with a larger sample. Along this line, an additional consideration is the sample size is relatively small (n = 42), and findings are correlational in nature - thus precluding ability to make causal attributions about changes in frontoamygdala connectivity and the experience of childhood trauma. Future longitudinal examinations of amygdala connectivity in larger samples will be necessary to better understand mechanisms contributing to neural circuit reorganization following early life stress. Finally, poor spatial resolution of standard fMRI acquisitions and susceptibility of the amygdala to EPI image distortions and draining vein effects (Merboldt et al., 2001) may lead to spatial localization errors. Furthermore, use of an 8 mm smoothing kernel to investigate small volumes such as amygdala subregions is not an optimal design. Despite these considerations, cytoarchitectonically-based amygdala FC analyses have yielded consistent, replicated
delineation of differential connectivity of major amygdala subregions (Etkin et al., 2009, Roy et al., 2009, Roy et al., 2013, Brown et al., 2014). Also, the fact that patterns observed here replicate prior neuroimaging and anatomical work in animals affords further confidence in the approach.

4.1 Conclusions

Reduced negative amygdala-sgACC connectivity was observed in a sociodemographic risk sample of youth exposed to severe trauma. Isolation of these effects augments prior studies in adults obtaining similar results by evaluating intrinsic connectivity in formative years (e.g., childhood) and by measuring change in a sample at substantially increased risk for developing clinical syndromes (i.e., urban, low income, minority). Our results support the supposition that the biological embedding of adversity in early life may include changes in neural connectivity, which in turn may alter interactions with the world and susceptibility to disease.
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References


Cicchetti D, Rogosch FA Equifinality and multifinality in developmental psychopathology.


Lieberman MD, Cunningham WA (2009) Type I and Type II error concerns in fMRI research: re-balancing the scale. Social cognitive and affective neuroscience 4:423-428.


