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Abnormal functional architecture of amygdala-centered networks in adolescent posttraumatic stress disorder

Abstract

Posttraumatic stress disorder (PTSD) is a prevalent, debilitating, and difficult to treat psychiatric disorder. Very little is known of how PTSD affects neuroplasticity in the developing adolescent brain. Whereas multiple lines of research implicate amygdala-centered network dysfunction in the pathophysiology of adult PTSD, no study has yet examined the functional architecture of amygdala subregional networks in adolescent PTSD. Using intrinsic functional connectivity analysis, we investigated functional connectivity of the basolateral (BLA) and centromedial (CMA) amygdala in 19 sexually abused adolescents with PTSD relative to 23 matched controls. Additionally, we examined whether altered amygdala subregional connectivity coincides with abnormal grey matter volume of the amygdaloid complex. Our analysis revealed abnormal amygdalar connectivity and morphology in adolescent PTSD patients. More specifically, PTSD patients showed diminished right BLA connectivity with a cluster including dorsal and ventral portions of the anterior cingulate and medial prefrontal cortices ($p < 0.05$, corrected). In contrast, PTSD patients showed increased left CMA connectivity with a cluster including the orbitofrontal and subcallosal cortices ($p < 0.05$, corrected). Critically, these connectivity changes coincided with diminished grey matter volume within BLA and CMA subnuclei ($p < 0.05$, corrected), with CMA connectivity shifts additionally relating to more severe PTSD symptoms. These findings provide unique insights into how perturbations in major amygdalar circuits could hamper fear regulation and drive excessive acquisition and expression of fear in PTSD. As such, they represent an important step towards characterizing the neurocircuitry of adolescent PTSD, thereby informing the development of reliable biomarkers and potential therapeutic targets.

Introduction

Posttraumatic stress disorder (PTSD) is a debilitating and difficult to treat psychiatric disorder, characterized by a constellation of symptoms related to the experience of a traumatic event (Patel, et al., 2012). Adolescent PTSD is of particular concern, as PTSD is more chronic and highly prevalent at this crucial developmental stage, coupled with increased risk for psychopathology later in life (Ackerman, et al., 1998; McLaughlin, et al., 2013; Perry and Azad, 1999). Critically, the perpetuating state of stress and anxiety in PTSD disrupts neuroplasticity in the developing adolescent brain, thereby hampering normal development of cognitive and emotional functions (Davidson and McEwen, 2012a; Lupien, et al., 2009). Yet, despite these concerns very little is known of how PTSD affects brain network organization in adolescents. Whereas multiple lines of research suggest amygdala subregional defects in the pathophysiology of adult PTSD (Brown, et al., 2014; Jovanovic and Ressler, 2010; Mahan and Ressler, 2012; Nicholson, et al., 2015; Patel, et al., 2012), no study has yet examined the functional architecture of amygdala subregional networks in adolescent PTSD. Knowledge on how major amygdalar circuits might be compromised in adolescent PTSD is crucial in gaining insight into the underlying pathophysiology, ultimately informing the development of reliable biomarkers and potential therapeutic targets.

The amygdala is a central component of brain’s affective processing system (LeDoux, 2000; Pessoa and Adolphs, 2010), and neurocircuitry models of PTSD consistently emphasize its central role in PTSD symptomatology (Patel, et al., 2012; Taghva, et al., 2013). Within these models, the amygdala is hyperresponsive to threat-related stimuli and acts as an overactive fear generator, in which its projections to memory circuits drive fear of trauma-related stimuli and context, while its projections to the brainstem, cerebellum and hypothalamus drive excessive fear responses and hyperarousal (Cisler, et al., 2014; Jovanovic and Ressler, 2010; Taghva, et al., 2013). Critically, the amygdala continues to drive excessive fear as medial prefrontal regions fail to downregulate amygdala hyperactivity (Jovanovic and Ressler, 2010; Taghva, et al., 2013). Within this amygdala-centered circuitry, the basolateral (BLA) and centromedial (CMA) subnuclei of the amygdala play imperative and distinctive roles in fear processing, through their unique connectivity profiles with cortical and subcortical territories (see Supplement 1 for schematic overview). The BLA receives information from multiple brain systems and is a site of integration with cortical areas, including those that regulate...
fear (Jovanovic and Ressler, 2010; LeDoux, 2007; Sah, et al., 2003). It is heavily involved in the perception, evaluation, and memory formation of emotionally salient stimuli (Davis and Whalen, 2001; LeDoux, 2007; McGaugh, et al., 1996). The CMA, in contrast, receives mostly modulatory inputs from the BLA and orbitofrontal cortex, and is less heavily innervated by sensory and associative regions (Barbas, et al., 2003; LeDoux, 2007; Sah, et al., 2003). It is the primary output site of the amygdala, and orchestrates fear responses via its projections to the brainstem, cerebellum and hypothalamus (Davis and Whalen, 2001; LeDoux, 2007). Despite the critical role of the amygdala in PTSD, very little is known of how BLA and CMA connectivity profiles may actually contribute to the pathophysiology of adolescent PTSD.

Most of our current knowledge about the distinctive functions and connectivity profiles of amygdala subregions stems from animal studies. However, recent advances in human neuroimaging have resulted in cytoarchitectonic probability maps of the amygdaloid complex (Amunts, et al., 2005), allowing to quantitatively map the unique connectivity profiles of amygdala subregions. Intrinsic functional connectivity (iFC) analysis, in particular, has proven a powerful method for delineating the functional architecture of intrinsically (i.e. spontaneously) coupled brain networks (Fox and Raichle, 2007). As such, dissociable connectivity profiles of the BLA and CMA were recently demonstrated in healthy adults and adolescents (Gabard-Durnam, et al., 2014; Roy, et al., 2009), consistent with earlier observations in rodents and primates (LeDoux, 2007; Sah, et al., 2003). These subregional connectivity profiles have been shown to reliably predict individual variations in anxiety in healthy adults (Li, et al., 2012), and in typically developing children as young as age nine (Qin, et al., 2014). Moreover, widespread disruption of both BLA and CMA connectivity profiles has been demonstrated in adult and adolescent generalized anxiety disorder, suggesting impairments in both the experience and regulation of emotions (Etkin, et al., 2009; Roy, et al., 2013). In parallel, disrupted BLA and CMA connectivity with regulatory prefrontal regions has been found in adult PTSD (Brown, et al., 2014; Nicholson, et al., 2015), while conjoint examination of amygdalar connectivity and morphology has been lacking with regard to PTSD. We addressed this critical gap by examining BLA and CMA intrinsic functional connectivity in a sample of adolescents with PTSD, relative to matched controls. Additionally, we employed voxel-based morphometry to examine whether alterations in grey matter volume of amygdala subregions coincide with abnormalities in BLA and CMA connectivity. Multimodal imaging of the amygdaloid complex may provide complementary information and novel insights on PTSD pathophysiology, which otherwise would only be partially revealed by each modality alone. Based on earlier work implicating amygdala-centered network dysfunction in abnormal fear processing and excessive fear responses (Barbas, 2007; Brown, et al., 2014; Cisler, et al., 2014; Etkin, et al., 2009; Jovanovic and Ressler, 2010; LeDoux, 2007; Pessoa and Adolphs, 2010; Roy, et al., 2013; Sah, et al., 2003; Shin and Liberzon, 2010), we hypothesized adolescents with PTSD to show diminished BLA connectivity with regulatory prefrontal regions, such as the medial prefrontal and anterior cingulate cortices. Moreover, we hypothesized adolescents with PTSD to show increased CMA connectivity with regions involved in fear expression. These include regions that modulate CMA activity (e.g. orbitofrontal cortex), as well as regions involved in the actual execution of fear responses (e.g. brainstem and hypothalamus). Additionally, given that abnormal connectivity and morphology of amygdala subregions tend to accompany each other in anxious individuals (Etkin, et al., 2009; Qin, et al., 2014), we hypothesized that abnormal BLA and CMA connectivity would coincide with altered grey matter volume of amygdala subregions. Finally, we expected that abnormal amygdalar connectivity would relate to more PTSD symptoms of stress and anxiety.
Method and Materials

Participants
Nineteen sexually abused adolescents with a DSM-IV diagnosis of PTSD (mean age = 16.16, SD = 1.79) and 23 age-, sex-, and IQ-matched healthy controls with no history of significant psychotrauma (mean age = 15.52, SD = 1.78) were selected, as part of the EPISCA study (Emotional Pathways’ Imaging Study in Clinical Adolescents). All PTSD patients had experienced repeated sexual abuse during their lifetime by one or more perpetrators in- or outside the family, and were referred for psychotherapy at an outpatient psychotrauma center. More details regarding participant inclusion are provided in Supplement 1.

Clinical Assessment
For all patients, after the clinical assessment by child and adolescent psychiatrists, DSM-IV diagnoses of PTSD were further assessed by trained clinical psychologists based on the child and parent versions of the Anxiety Disorders Interview Schedule (ADIS) (Silverman, 1996), a diagnostic tool for obtaining DSM-IV-based classifications of anxiety disorders. Following this, additional clinical measures were used to assess the severity of PTSD and related internalizing symptoms. These included the Trauma Symptom Checklist for Children (TSCC) (Briere, 1996), the Adolescent Dissociative Experiences Scale (A-DES) (Armstrong, et al., 1997), and the Children’s Depression Inventory (CDI) (Kovacs, 1992). Though these questionnaires are not typically used for diagnostic purposes, clinical cut-off scores have been suggested. For the TSCC a mean total score of ≥ 60 suggests acute and chronic posttraumatic symptomatology (Briere, 1996), with mean scores of ≥ 4.0 on the A-DES suggesting pathological dissociation (Armstrong, et al., 1997), and mean CDI scores of ≥ 16 indicating depressive symptomatology (Kovacs, 1992). More detailed description of these questionnaires is provided in Supplement 1. The same measures were also applied for the control group, and control participants were excluded when they fulfilled the criteria for a DSM-IV diagnosis or had (sub)clinical scores on clinical questionnaires.

Data Acquisition and Preprocessing
Resting-state (RS) fMRI data were collected using a Philips 3T Achieva MRI scanner (Philips Healthcare, Best, The Netherlands) with an 8-channel SENSE (Sensitivity Encoding) head coil. Prior to scanning, all participants were accustomed to the scanning situation by lying in a dummy scanner and hearing scanner sounds. Participants were instructed to lie still with their eyes closed and not to fall asleep during the 6-minute RS scan. More detail regarding data acquisition is provided in Supplement 1.

All data were preprocessed and analyzed using FSL (http://fsl.fmrib.ox.ac.uk/fsl/fsl-wiki/) version 5.0.1. Preprocessing consisted of (1) nonbrain-tissue removal, (2) motion correction, (3) grand mean-based intensity normalization of the entire 4-D data set by a single scaling factor, (4) slice timing correction, (5) spatial smoothing with a 6 mm full width at half maximum Gaussian kernel, and (6) temporal bandpass filtering at $0.009 < f < 0.15$ Hz, which improves BOLD signal estimation and produces connectivity patterns that relate most closely to task-based activations (Biswal, et al., 1995; Fox and Raichle, 2007; Fransson, 2006; Roy, et al., 2009; Toro, et al., 2008). Finally, the RS data were registered to T1-weighted anatomical images, and subsequently to the 2-mm Montreal Neurological Institute (MNI) standard space image (Roy, et al., 2013; Roy, et al., 2009). The maximum allowable mean displacement due to excessive head motion was set at 3 mm translation or 3° rotation in any direction. Additionally, to guard against the effects of in-scanner micro-motion on connectivity patterns we implemented motion-censoring, also known as “scrubbing” (Power, et al., 2012; Satterthwaite, et al., 2013) (see Supplement 1 for details).

Region of Interest Definition
Using cytoarchitectonic probabilistic maps of amygdala subnuclei provided in FSL’s Juelich histological atlas (Amunts, et al., 2005), BLA and CMA region of interest (ROI) masks were created in both hemispheres (see Supplement 1). These probability maps have been validated in pediatric postmortem studies (Kim, et al., 2010), and have proven highly reliable and accurate in guiding amygdala parcellation in pediatric populations (Gabard-Durnam, et al., 2014; Qin, et al., 2014; Qin, et al., 2012; Roy, et al., 2013). In line with recent developmental studies (Qin, et al., 2014; Qin, et al., 2012), voxels were included in the ROI masks only if the probability of their assignment to the BLA or CMA was higher than any other nearby structures with greater than 40% likelihood. Each voxel was exclusively assigned to only one region, and overlapping voxels were assigned to the region that had the greatest probability (Qin, et al., 2014; Qin, et al., 2012). These masks were used in subsequent iFC and structural analyses.
Functional Connectivity Analysis
Seed-based whole brain iFC analysis was employed to reveal BLA and CMA connectivity (Fox and Raichle, 2007). For each hemisphere, a general linear model was created that included individual participant's mean time series of both the BLA and CMA subnuclei as predictors (Roy, et al., 2013; Roy, et al., 2009). Signal from the white matter and cerebrospinal fluid (see Supplement 1 for details), six motion parameters, and parameters obtained from the motion censoring procedure (see Supplement 1 for details) were temporally filtered and included in this model as covariates of no interest to correct for physiological and motion-related noise. This resulted in individual subject–level maps of all voxels uniquely exhibiting iFC with each amygdala subdivision, accounting for the relationships of the other subdivision (Roy, et al., 2013). Subject-level iFC maps of the BLA and CMA were fed into a higher-level mixed-effects group analysis, with age, sex, and IQ (demeaned across groups) included as covariates of no interest. Resulting statistical maps were corrected for multiple comparisons using cluster-based correction ($p<0.05$, initial cluster forming threshold $Z>2.3$). Additionally, similar to Qin et al. (Qin, et al., 2012), spatial correlations were computed between each participant’s BLA and CMA connectivity maps to quantify the overall similarity between BLA and CMA target networks (see Supplement 1 for details). This provides important complementary information about the functional differentiation and segregation of BLA and CMA networks (Qin, et al., 2012). Previous studies have shown that abnormal anxiety not only impacts the connectivity strength of the BLA and CMA with their respective targets, but also shapes the functional differentiation between their connectivity profiles (i.e., increased overlap between BLA and CMA networks) (Etkin, et al., 2009; Roy, et al., 2013). Finally, exploratory conjunction analyses examined whether PTSD may affect common connectivity patterns of BLA and CMA subregions (see Supplement 1 for details).

Grey Matter Volume Analysis
To examine whether alterations in grey matter volume of amygdala subregions may coincide with abnormalities in BLA and CMA connectivity, optimized voxel-based morphometry (VBM) was performed. Grey matter volume was analyzed using FSL’s VBM tool (Douaud, et al., 2007). In brief, structural images were grey matter-segmented, a study-specific grey matter template was created, and all native-space grey matter images were registered to this template. Finally, between groups voxelwise permutation-based nonparametric testing of grey matter volume was restricted to the BLA and CMA subnuclei and corrected for multiple comparisons, using Threshold-Free Cluster Enhancement (TFCE) with Family-Wise Error (FWE) correction ($p<0.05$). Age, sex, and IQ (demeaned across groups) were included in the analyses as covariates to correct for their possible confounding effects. More details regarding the VBM analysis are provided in Supplement 1.

Functional Connectivity and Structure
To examine whether coinciding changes in amygdalar connectivity and structure are associated with each other, partial correlation analyses were performed. More specifically, patients’ mean grey matter volume and connectivity strength within regions of significant group differences were fed into a partial correlation model, adjusted for age, sex, and IQ.

Functional Connectivity and Symptom Severity
Partial correlation analyses in PTSD patients (adjusted for age, sex, and IQ) examined the association between subregional connectivity strength (i.e., mean $Z$ values) within areas of significant group differences and PTSD symptoms of stress and anxiety (as measured with posttraumatic stress and anxiety subscales of the TSCC). For transparency, similar analyses in the PTSD group examined whether amygdala connectivity changes may also relate to depressive symptomatology (as measured with the CDI).

Structural Integrity and Symptom Severity
Though not a primary objective of this study, partial correlation analyses in PTSD patients (adjusted for age, sex, and IQ) examined the association between subregional volumetrics within areas of significant group differences and PTSD symptoms of stress and anxiety (i.e., TSCC). Likewise, similar analyses in the PTSD group examined whether volumetric changes within amygdala subregions may also relate to depressive symptoms (i.e., CDI).

PTSD Duration and Amygdalar Connectivity and Structure
As PTSD duration has been proposed to impact brain structure and function (Bremner, 2006), exploratory analyses examined the association between PTSD duration and amygdalar connectivity and structure. Specifically, partial correlation analyses in PTSD patients...
(adjusted for age, sex, and IQ) examined the association between PTSD duration and subregional connectivity and volumetrics within areas of significant group differences.

Effects of Comorbidity and Medication Use on Functional Connectivity
Similar to Roy et al. (Roy, et al., 2013) and Cullen et al. (Cullen, et al., 2014), we performed post-hoc analyses to examine the effects of comorbidity and medication use on iFC. Using mean Z values representing connectivity strength within regions of significant group differences, multivariate analyses of variance (MANOVA) were conducted to compare adolescents with PTSD (excluding either those with a comorbid disorder, those using medication, or both) to healthy adolescents. Additionally, we compared adolescent PTSD patients with a comorbid disorder to those without, while also comparing patients who were on medication to those who were not.

Results

Sample Characteristics
As shown in Table 1, the matched groups did not differ with respect to age, sex, and IQ. Importantly, the groups also did not differ on measures of head movement during RS data acquisition. As expected, clinical measures revealed more PTSD, anxiety, and depressive symptoms in patients (Table 1). The patient group comprised 19 adolescents with a DSM-IV diagnosis of PTSD, with mean illness duration of 5.23 years (SD = 3.58). Eight patients had a secondary comorbid disorder (depressive disorder N = 7, attention deficit hyperactivity disorder N = 1). Most patients were treatment-naïve, with only three patients taking psychotropic medication. All PTSD patients had experienced serious and longstanding sexual abuse during their lifetime, including repeated or group rape, by one or more perpetrators in- or outside the family. In 77.8% of the cases this was by another person than an attachment figure. Sexual abuse was reported to the police in 60.9% of the cases, child welfare was involved in 56.5% of the cases, while 17.4% had a child protection measure (family custody). None of the participating control adolescents had experienced significant psychotrauma, and were not involved with police, child welfare or child protection.
Functional Connectivity Analysis

Across groups, whole-brain iFC analysis revealed dissociable BLA and CMA connectivity profiles with a distributed set of cortical and subcortical regions, consistent with established models of amygdaloid circuitry (LeDoux, 2007; Qin, et al., 2012; Roy, et al., 2013; Roy, et al., 2009; Sah, et al., 2003) (Figure 1). Moreover, spatial similarity analysis of each participant’s BLA and CMA connectivity maps revealed negative spatial correlations between BLA and CMA target networks (mean $r = -0.23$, $p<0.001$), thus corroborating the distinctive and divergent connectivity profiles of the BLA and CMA in a quantitative manner (Figure 1). As shown in Figure 2, direct group comparison of iFC revealed that PTSD patients had diminished right BLA connectivity with a cluster including dorsal and ventral portions of the anterior cingulate (ACC) and medial prefrontal (PFC) cortices (see Table S1 in Supplement 1 for complete results). In contrast, PTSD patients showed increased left CMA connectivity with a cluster including the orbitofrontal and subcallosal cortices (Figure 3) (see Table S2 in Supplement 1 for complete results). Notably, group comparison of spatial similarity between BLA and CMA target networks revealed no significant group differences in the right ($t(40) = -0.04$, $p = 0.97$) or left ($t(40) = 0.40$, $p = 0.69$) hemisphere (Figure 1). Thus, although the connectivity strength of the BLA and CMA with their respective targets is altered in adolescent PTSD, the functional differentiation and segregation of BLA and CMA networks is largely maintained.

Figure 1. Dissociable connectivity profiles of BLA and CMA complexes. (A) Lateral and medial views of the BLA (blue) and CMA (red) target networks in controls and adolescent PTSD patients. Overlap between BLA and CMA networks is shown in purple. Images are in radiological convention (i.e. right is left and vice versa). (B) Bar graph showing spatial correlations between BLA and CMA networks in controls and patients. Positive values indicate similarities while negative values indicate differences between BLA and CMA networks. Group comparison of spatial similarity between BLA and CMA target networks revealed no significant group differences.
Figure 2. Abnormal right BLA connectivity in adolescent PTSD patients. (A) Medial and anterior views of right BLA connectivity in controls (first row) and adolescent PTSD patients (second row). Direct group comparison revealed that PTSD patients had diminished right BLA connectivity with a cluster including dorsal and ventral portions of the anterior cingulate and medial prefrontal cortices (third row). (B) Representative sagittal and axial slices of between group differences in right BLA connectivity, along with a bar graph showing lower mean connectivity strength (i.e. mean $Z$) in PTSD patients within the displayed cluster. Statistical maps are corrected for multiple comparisons at the cluster level ($Z>2.3, p<0.05$), and brain images are in radiological convention (i.e. right is left and vice versa).

Figure 3. Abnormal left CMA connectivity in adolescent PTSD patients. (A) Medial and ventral views of left CMA connectivity in controls (first row) and adolescent PTSD patients (second row). Direct group comparison revealed that PTSD patients had increased left CMA connectivity with a cluster including the orbitofrontal and subcallosal cortices (third row). Red circles mark the effect site. (B) Representative coronal and axial slices of between group differences in left CMA connectivity, along with a bar graph showing higher mean connectivity strength (i.e. mean $Z$) in PTSD patients within the displayed cluster. Statistical maps are corrected for multiple comparisons at the cluster level ($Z>2.3, p<0.05$), and brain images are in radiological convention (i.e. right is left and vice versa). (C) Stronger CMA connectivity with the orbitofrontal/subcallosal region related to more symptoms of stress and anxiety in PTSD patients ($r = 0.59$ and $0.66$; both $p<0.05$). Individual patient’s mean connectivity strength plotted against their symptom severity scores visualizes the direction of the association. PTSD-related stress and anxiety was measured with the posttraumatic stress and anxiety subscales of the Trauma Symptom Checklist for Children (TSCC).
Grey Matter Volume Analysis
To examine whether alterations in grey matter volume of amygdala subregions may coincide with abnormalities in BLA and CMA connectivity, optimized VBM analysis was performed. As shown in Figure 4, our analysis revealed diminished grey matter volume in right BLA and CMA in PTSD patients ($p<0.05$, TFCE and FWE-corrected). Our analysis also revealed diminished grey matter volume in the left CMA in PTSD patients, albeit uncorrected for multiple comparisons ($p<0.05$, TFCE-corrected). Our structural analysis thus suggests that intra-amygdalar abnormalities in grey matter volume coincide with amygdalar network dysfunction in adolescent PTSD.

Functional Connectivity and Structure
To examine whether coinciding changes in amygdalar connectivity and structure are associated with each other, partial correlation analyses were performed. Although abnormal connectivity and morphology of amygdala subregions coincided in PTSD patients, we found no significant associations between them ($p's>0.05$).

Functional Connectivity and Symptom Severity
Partial correlation analyses in patients examined the association between subregional connectivity strength within areas of significant group differences and PTSD symptoms of stress and anxiety. Our analyses revealed that stronger CMA connectivity with the orbitofrontal/subcallosal region related to more symptoms of stress and anxiety in PTSD patients ($r = 0.59$ and $0.66$; both $p<0.05$) (Figure 3). Such association was not found for BLA-medial prefrontal connectivity ($p's>0.05$), which could be due to lack of statistical power and a possible ceiling effect in our relatively small sample of PTSD patients. In addition, no association was observed between amygdala subregional connectivity changes and depressive symptomatology in PTSD patients ($p's>0.05$), suggesting that connectivity changes documented here might potentially be more characteristic of PTSD than depressive symptoms.

Structural Integrity and Symptom Severity
For transparency, partial correlation analyses in patients examined the association between subregional volumetrics within areas of significant group differences and PTSD symptoms of stress and anxiety. The analysis revealed no significant associations between structural integrity of amygdala subregions and PTSD symptomatology ($p's>0.05$). Likewise, no associations were found between structural integrity of amygdala subregions and depressive symptoms ($p's>0.05$).

PTSD Duration and Amygdalar Connectivity and Structure
Exploratory partial correlation analyses in PTSD patients examined the association between PTSD duration and subregional connectivity and volumetrics within areas of significant group differences. The analyses revealed that neither connectivity nor structure...
of the amygdaloid complex is associated with chronicity of PTSD symptoms ($p>0.05$).
While this could relate to lack of statistical power and possibly a ceiling effect, it may also
suggest that functional and structural changes we documented might be more represent-
ative of PTSD vulnerability and diagnosis rather than its duration.

**Effects of Comorbidity and Medication Use on Functional Connectivity**

Post-hoc analyses examined the effects of comorbidity and medication use on group
differences in iFC, per Roy *et al.* (Roy, et al., 2013) and Cullen *et al.* (Cullen, et al., 2014).
Our analysis revealed that all group differences in iFC remained significant, while excluding
patients with a secondary comorbid disorder ($N=8$ excluded) (multivariate model: $F(4,29)=19.3$, $p<0.001$; right BLA: $F(1,32)=13.2$, $p<0.001$; left CMA: $F(1,32)=23.7$, $p<0.001$). Likewise, group differences in iFC remained significant, while excluding patients using medication ($N=3$ excluded) (multivariate model: $F(4,34)=21.78$, $p<0.001$; right BLA: $F(1,37)=19.8$, $p<0.001$; left CMA: $F(1,37)=15.4$, $p<0.001$). A final analysis excluding both patients with a secondary comorbid disorder and patients taking medication was not necessary, as only patients with a comorbid disorder were on medication. Additionally, we found no significant differences in iFC between PTSD patients with a comorbid disorder relative to those without (multivariate model: $F(4,14)=0.98$, $p=0.45$; right BLA: $F(1,17)=0.31$, $p=0.87$; left CMA: $F(1,17)=1.56$, $p=0.23$), or between patients who were on medication relative to those who were not (multivariate model: $F(4,14)=0.76$, $p=0.57$; right BLA: $F(1,17)=0.80$, $p=0.38$; left CMA: $F(1,17)=0.79$, $p=0.38$).

**Discussion**

The current study uniquely investigated the intrinsic functional architecture of amygdala-centered networks in sexually abused adolescents with PTSD, relative to matched controls. We hypothesized altered BLA and CMA functional connectivity in PTSD patients, coupled with abnormalities in grey matter volume of amygdala subregions. In line with our hypotheses, we found evidence for altered BLA and CMA connectivity with regulatory prefrontal regions in PTSD patients. Critically, these connectivity changes coincided with diminished grey matter volume within BLA and CMA subnuclei, with CMA connectivity shifts additionally relating to more severe symptoms of PTSD. To our knowledge no previous study has characterized the emergence of such functional and structural abnormalities in severely traumatized adolescents. These findings therefore provide new insights into how perturbations in major amygdalar circuits could hamper fear regulation and drive excessive acquisition and expression of fear in adolescent PTSD.

**Amygdala Functional Connectivity**

In line with neurocircuitry models of PTSD (Jovanovic and Ressler, 2010; Patel, et al., 2012; Taghva, et al., 2013), adolescent PTSD patients showed diminished right BLA connectivity with a cluster including dorsal and ventral portions of the anterior cingulate (ACC) and medial prefrontal (PFC) cortices. These interconnected medial prefrontal structures serve myriad functions, but they also play imperative and distinctive roles at various stages of fear processing through their unique relationships with the BLA and other brain regions (Etkin, et al., 2011). The dorsal ACC and adjacent dorsomedial PFC connect primarily to cognitive brain regions such as the lateral PFC, and are active in concert with the BLA during appraisal, acquisition, and cognitive regulation of fear (Chiba, et al., 2001; Etkin, et al., 2011; Vidal-Gonzalez, et al., 2006). In contrast, the ventral ACC and adjacent ventromedial PFC connect directly and reciprocally to affective brain regions such as the BLA and hippocampus, and are mainly involved in automatic regulation and extinction of fear (Chiba, et al., 2001; Etkin, et al., 2011; Etkin, et al., 2006; Milad and Quirk, 2002; Myers-Schulz and Koenigs, 2012; Vidal-Gonzalez, et al., 2006). In fact, feed-forward inhibitory projections from these ventral medial prefrontal areas to the BLA are deemed fundamental in regulating and extinguishing fear, as they compete with the fear pathway represented within the BLA-to-CMA microcircuit (Milad and Quirk, 2002; Peters, et al., 2009; Vidal-Gonzalez, et al., 2006). Noteworthy, this ventral inhibitory pathway also allows top-down governance of amygdala by dorsal ACC and dorsomedial PFC regions that lack direct connections to the amygdaloid complex (Schiller and Delgado, 2010). Diminished BLA connectivity with medial prefrontal areas as reported here could thus reflect a general dysfunction in fear processing, in which both the appraisal and acquisition of fear, as well as the effortful (i.e. dorsal) and automatic (i.e. ventral) regulation of fear are perturbed. This
notion fits well with the clinical presentation of PTSD (Bradley, et al., 2011; Ehring and Quack, 2010), and is supported by a wealth of data consistently linking abnormal experience and regulation of fear to disrupted functional integrity within the amygdala-medial prefrontal circuit (Brown, et al., 2014; Eskin, et al., 2006; Etkin and Wager, 2007; Jin, et al., 2013; Milad and Quirk, 2002; Myers-Schulz and Koenigs, 2012; Shin and Liberzon, 2010; Sripada, et al., 2012; Stevens, et al., 2013; Vidal-Gonzalez, et al., 2006; Wolf and Herringa, 2015). In fact, perturbed amygdala-medial prefrontal coupling is deemed central to the pathophysiology of PTSD, as it promotes amygdala hyperactivity and diminished medial prefrontal control, thus increasing the propensity for excessive fear (Etkin and Wager, 2007; Jovanovic and Ressler, 2010). As such, our finding provides novel evidence that disrupted BLA-medial prefrontal connectivity might be a reliable neural marker of PTSD and a prominent feature of pediatric PTSD.

In line with recent work on the neurocircuitry of fear and anxiety (Milad and Rauch, 2007; Myers-Schulz and Koenigs, 2012; Rempel-Clower, 2007; Shin and Liberzon, 2010), adolescent PTSD patients also showed increased left CMA connectivity with a cluster including the orbitofrontal and subcallosal cortices. This increase in CMA connectivity was additionally related to more stress and anxiety symptoms in PTSD patients. The neighboring and highly interconnected orbitofrontal and subcallosal cortices serve numerous functions, but they also play a critical role in behavioral and physiological aspects of fear processing by modulating CMA activity (Barbas, 2007; Barbas, et al., 2003; Hamani, et al., 2011; Sah, et al., 2003). Such modulation of fear processing occurs mainly via orbitofrontal projections onto the CMA complex. Heavy projections from the posterior orbitofrontal cortex target the CMA via the intercalated masses, a subpopulation of amygdalar neurons sending feed-forward inhibitory inputs to the CMA complex (Barbas, 2007). The majority of CMA neurons are GABAergic (Pitkanen and Amaral, 1994; Saha, et al., 2000), sending inhibitory projections to central autonomic structures such as the brainstem and hypothalamus (Barbas, 2007). As such, orbitofrontal inhibition of the CMA via intercalated masses will thus result in disinhibition of central autonomic structures and produce a state of heightened vigilance and fear (Barbas, 2007). We thus tentatively conclude that exaggerated CMA connectivity with orbitofrontal and subcallosal cortices could drive the perpetuating state of hypervigilance and fear often reported in patients with PTSD. In support of this notion, stronger CMA connectivity with the orbitofrontal/subcallosal region related to more stress and anxiety in our sample of adolescents with PTSD. Excessive fear and anxiety do indeed involve hyperactivity and hyperconnectivity within the amygdala-orbitofrontal/subcallosal circuitry (Andreescu, et al., 2014; Barbas and Zikopoulos, 2007; Gold, et al., 2014; Holz, et al., 2014; Milad and Rauch, 2007; Myers-Schulz and Koenigs, 2012; Phillips, et al., 2003; Sladky, et al., 2013), and selective lesions within this circuitry significantly reduce the behavioral and physiological parameters of fear (Izquierdo, et al., 2005; Kalin, et al., 2007; Vasa, et al., 2004). However, our finding may also relate to augmented fear learning in PTSD, as recent data suggests a crucial role for the CMA not only in the expression but also the acquisition of fear. For instance, pre-training lesioning of the CMA in rodents impairs the acquisition of fear, while post-training lesions of the CMA prevent the expression of leaned fear responses (Ciocchi, et al., 2010a; Wilensky, et al., 2006; Zimmerman, et al., 2007). Moreover, animals with BLA lesions still demonstrate fear learning and this capacity is believe to be mediated by the CMA (Goosens and Maren, 2003). In sum, our CMA findings document in humans what was previously established in animal studies, linking perturbations in the CMA-orbitofrontal/subcallosal circuitry to abnormal acquisition and expression of fear.

Although the connectivity strength of the BLA and CMA with their respective targets was altered in adolescent PTSD patients, spatial similarity analysis revealed that BLA and CMA connectivity profiles in patients are essentially as segregated and divergent as in healthy controls. In other words, adolescent PTSD mainly affected the connectivity strength within BLA and CMA networks, while sparing the functional differentiation between them. A similar pattern was recently demonstrated in adult PTSD patients (Brown, et al., 2014), which further supports this notion. Our findings thus confirm the distinctive and divergent connectivity profiles of the BLA and CMA (LeDoux, 2007; Qin, et al., 2012; Roy, et al., 2013; Roy, et al., 2009; Sah, et al., 2003), and suggest unique relationships between these amygdalar nuclei and regulatory prefrontal regions in the pathophysiology of adolescent PTSD. To our knowledge no previous study has characterized the emergence of such a pattern in severely traumatized adolescents. Converging lines of evidence suggest that experience-dependent neuroplasticity in the developing adolescent brain plays a critical role in modulating the development of functional brain networks (Paus, 2005;
Toga, et al., 2006). Moreover, animal data shows that stress and anxiety induce long-lasting changes in amygdala structure and connectivity (Lupien, et al., 2009). We thus suggest that abnormal stress and anxiety early in life may cause the reconfiguration of amygdalar networks reported here and exacerbate vulnerability for stress-related psychopathology.

It is worth mentioning that amygdalar connectivity changes we documented seemed somewhat lateralized, with PTSD patients exhibiting diminished right BLA and amplified left CMA coupling with regulatory frontal regions. The lateralization of amygdala function, especially of its different subregions, remains a topic of intense debate (Baas, et al., 2004; Sergerie, et al., 2008b; Styliadis, et al., 2014). Some theories opine that right amygdala mediates relatively global and transient emotional responses, while its left counterpart seems to serve more specific and sustained forms of emotional responding (Baas, et al., 2004; Sergerie, et al., 2008b). Crucially, recent data suggests that right BLA encodes precise affective features (e.g., punishment), while left CMA seems to process general affective valence (e.g., good vs. bad) (Styliadis, et al., 2014). One may thus speculate that the lateralized connectivity changes we seem to document could be indicative of a general dysfunction in affective processing among patients with PTSD. Although PTSD and anxiety seem to prompt lateralized changes in BLA and CMA connectivity (Brown, et al., 2014; Nicholson, et al., 2015; Roy, et al., 2013), we do express our reservations on whether the apparent laterality of current and previous findings reflects genuine differences in affective processes. Clearly, additional studies are needed to further investigate the question of laterality in intrinsic connectivity of amygdala subregions in patients with PTSD.

**Amygdala Structure**

In conjunction with abnormal amygdalar connectivity, structural analysis revealed diminished grey matter volume of BLA and CMA subnuclei in adolescent patients with PTSD. This corroborates previous reports in PTSD (Depue, et al., 2014; Karl, et al., 2006; Mollica, et al., 2009; Morey, et al., 2012; Rogers, et al., 2009; Veer, et al., 2015; Weems, et al., 2013), and is in line with studies linking smaller amygdala volumes to enhanced fear conditioning and exaggerated stress reactivity (Gianaros, et al., 2008; Hartley, et al., 2011; Maroun, et al., 2013; Yang, et al., 2008), two prominent features of PTSD. For instance, strains of mice with relatively smaller amygdala volumes exhibit stronger fear conditioning and greater corticosterone responses to stress than mice with larger amygdala volumes (Yang, et al., 2008). Similarly, enhanced fear acquisition and stress reactivity, as measured by skin conductance and mean arterial pressure respectively, correlate with smaller amygdala volumes in human beings (Gianaros, et al., 2008; Hartley, et al., 2011). Our finding further links diminished amygdala volume to abnormal stress and anxiety, but also suggests that this intra-amygdalar abnormality coincides with amygdalar network dysfunction in PTSD. Following this perspective, healthy individuals with genetic susceptibility for stress-related psychopathology show disrupted amygdala-prefrontal functional connectivity along with reduced amygdala volumetrics (Pezawas, et al., 2005). Additionally, highly anxious individuals show abnormal functional connectivity of amygdala subregions accompanied by changes in subregional volumetrics of the amygdaloid structure (Etkin, et al., 2009; Qin, et al., 2014). Here we further implicate coinciding changes in amygdalar connectivity and structure in the pathophysiology of abnormal anxiety. Similar to previous reports though (Etkin, et al., 2009; Pezawas, et al., 2005; Qin, et al., 2014), we found no significant correlation between these functional and structural alterations. While this could relate to lack of statistical power and a possible ceiling effect, it may also be suggestive of a complex structure-function relationship involving multiple moderating and mediating factors. The exact mechanism by which changes in structure may impact human brain function is still poorly understood. However, animal studies suggest that cellular changes in grey and white matter, including axon sprouting, dendritic arborization and fiber organization, could impact network communication and information processing (Zatorre, et al., 2012). Future advances in imaging technology, and greater dialogue between human neuroimaging and cellular/molecular neuroscience, could further our understanding of the complex interplay between brain structure and function.

Developmental data shows that abnormal stress and anxiety early in life cause greater dendritic arborization and increased synaptogenesis, leading to an initial increase in amygdala growth and activity (Davidson and McEwen, 2012b; Tottenham and Sheridan, 2009; Vyas, et al., 2006). However, a prolonged period of stress-induced increase in amygdala growth and activity promotes glucocorticoid receptor hypersensitivity and overexposure to stress hormones (i.e., neurotoxicity), eventually causing neural atrophy and even cell death (Hanson, et al., 2014; Teicher, et al., 2003; Tottenham and Sheridan, 2009;
This study preceded or followed the onset of PTSD. Additionally, the small number of adolescents in our sample may to some extent limit the generalizability of our results. Longitudinal research in larger samples with a more balanced male to female ratio could tackle these limitations. In line with other PTSD studies, some of our patients had a secondary comorbid depressive disorder, and this may potentially affect the specificity of our results. Such comorbidity, however, is deemed a typical element of clinical PTSD, and exclusion of these patients would have resulted in a highly atypical sample lacking external validity (Morey, et al., 2012; Shin and Liberzon, 2010; Zahn-Waxler, et al., 2000). As such, we performed post-hoc analyses but found no impact of comorbidity on amygdala iFC. Most of our patients were treatment-naïve, with only 3 patients taking psychotropic medication. While in potential this may marginally affect our findings, post-hoc analyses showed no impact of medication use on amygdala iFC reported here. Importantly, leading researchers in the field of PTSD neuroimaging strongly argue against exclusion of medicated patients from studies, as this may limit the generalizability of the findings (Lanius, et al., 2010). It should be noted that we did not include control participants with a history of trauma exposure resembling that of the PTSD patients. However, sexually abused adolescents without any symptomatology and not in need of any psychological help are often reluctant to participate in such studies, owing to feelings of shame and embarrassment associated with their trauma. We are therefore unable to fully ascertain whether trauma exposure itself, regardless of diagnostic status, is associated with amygdalar abnormalities reported here. Nonetheless, our findings merit attention as the first evidence for perturbed function and structure of amygdala subregions in traumatized adolescents with PTSD diagnosis. The findings provide an interesting focus for future research, which should further explore and validate amygdalar abnormalities reported here. Finally, physiological fluctuations (heart rate and respiration) were not recorded during resting-state data acquisition. Although we applied temporal filtering and regressed out white matter and cerebrospinal fluid signal to eliminate physiological noise (Fox and Raichle, 2007; Hallquist, et al., 2013; Windischberger, et al., 2002), these physiological fluctuations may still have been a source of noise influencing our data.

Notwithstanding these limitations, our study has several strengths that increase the reliability of our findings. First, to allow more accurate group comparison of amygdalar networks, patients and healthy controls were matched for age, sex, and IQ, while including these variables in the analyses as covariates to account for their possible confounding effects. Second, we included a highly selected and homogenous patient group with regard to type of trauma and chronicity of PTSD, which further strengthens the reliability of our findings. Third, partitioning the amygdala into BLA and CMA complexes produced dissociable connectivity profiles that were consistent with established models of amygdaloid circuitry. As such, we were able to demonstrate anxiety-related perturbations in amygdala subregional connectivity, consistent with what was previously established in animal studies. Finally, multimodal imaging of the amygdaloid complex in the present study produced complementary and novel results, which otherwise would only be partially detected by each modality alone.

Conclusions
In summary, adolescent PTSD patients showed abnormal amygdala subregional connectivity with regulatory prefrontal regions, coupled with diminished grey matter volume of amygdala subregions. These findings provide unique insights into how perturbations in major amygdalar circuits could hamper fear regulation and drive excessive acquisition and expression of fear in PTSD. As such, they represent an important step towards characterizing the neurocircuitry of adolescent PTSD, thereby informing the development of reliable biomarkers and potential therapeutic targets. For a deeper understanding of PTSD pathophysiology, it would be important to examine whether functional and structural integrity of amygdala subregions predict susceptibility, chronicity, and treatment response in PTSD.

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Financial disclosure

The authors declare no conflict of interest.
Supplementary Materials and Methods

Participants
Nineteen sexually abused adolescents with a DSM-IV diagnosis of PTSD (mean age = 16.16, SD = 1.79) and 23 age-, sex-, and IQ-matched healthy controls (mean age = 15.52, SD = 1.78) were selected, as part of the EPISCA study (Emotional Pathways’ Imaging Study in Clinical Adolescents). EPISCA is a longitudinal MRI study in which sexually abused adolescents with PTSD and healthy controls were followed over a six-month period (January 2010 till August 2012). The PTSD group underwent an MRI scanning protocol prior to the start of their regular cognitive behavioral therapy (CBT). Healthy control adolescents were recruited through local advertisement. The current study reports on cross-sectional MRI data from both groups.

Inclusion criteria for the patient group were: having experienced repeated sexual abuse during their lifetime by one or more perpetrators in- or outside the family, DSM-IV diagnosis of PTSD, and being referred for CBT at an outpatient psychotrauma center. Inclusion criteria for the control group were: no current or past DSM-IV diagnoses of axis I and/or axis II disorders, no clinical scores on validated mood and behavioral questionnaires, no history of significant psychotrauma, and no current psychotherapeutic and/or psychopharmacological intervention of any kind. Exclusion criteria for all participants were: (1) primary DSM-IV diagnosis of oppositional defiant disorder, conduct disorder, pervasive developmental disorders, Tourette’s syndrome, obsessive-compulsive disorder, bipolar disorder, and psychotic disorders, (2) current substance abuse, (3) a history of neurological disorders or severe head injury, (4) age <12 or >20 years, (5) pregnancy, (6) left-handedness, (7) IQ score <80, as measured by either the Wechsler Intelligence Scale for Children (Wechsler, 1991) or the Wechsler Adult Intelligence Scale (Wechsler, 1997), and (8) general MRI contraindications (e.g., metal implants, claustrophobia). Participants were scanned within two weeks of initial screening, and all were new to MRI scanning procedures. The medical ethics committee of the Leiden University Medical Center approved the study and written informed consent was obtained from all adolescents and their parents.

From the original group of 53 adolescents (21 patients, 32 controls), eleven were excluded from the current resting-state (RS) fMRI study due to image artifacts and group-wise matching for age, sex and IQ. Eventually, 42 participants (19 patients, 23 controls) aged 12 to 19 years (M = 15.81, SD = 1.79), were included in the MRI analysis.

Clinical Measures
The TSCC is a self-report questionnaire with 54 items evaluating posttraumatic symptomatology in adolescents, and is scored on a 4-point Likert scale describing the severity of symptoms (0 = absence of symptomatology to 3 = severe symptomatology). The A-DES is a self-report questionnaire with 30 items evaluating dissociation in adolescents, and is scored on an 11-point Likert scale describing the frequency of symptoms (0 = never to 10 = always). The CDI is a self-report questionnaire with 27 items that correspond with DSM-IV dimensions of depressive disorders in adolescents, and is scored on a 3-point Likert scale describing the severity of symptoms (0 = absence of symptomatology to 2 = severe symptomatology).

Data Acquisition
Resting-state (RS) fMRI data were collected using a Philips 3.0T Achieva MRI scanner (Philips Healthcare, Best, The Netherlands) with an 8-channel SENSE (Sensitivity Encoding) head coil. Prior to scanning, all participants were accustomed to the scanning situation by lying in a dummy scanner and hearing scanner sounds. For RS data acquisition, participants were instructed to lie still with their eyes closed and not to fall asleep. A total of 160 T2*-weighted gradient-echo echo-planar imaging (EPI) volumes were acquired, using the following scan parameters: repetition time (TR) = 2200 ms, echo time (TE) = 30 ms, flip angle = 80°, 38 transverse slices with an in-plane voxel resolution of 2.75x2.75 mm, 2.72 mm slice thickness, field of view (FOV) = 220x220 mm. The total RS acquisition time was 6 minutes. For anatomical reference and grey matter analysis, a T1-weighted anatomical scan was acquired for each participant with the following scan parameters: TR = 9.7 ms, TE = 4.6 ms, 140 sagittal slices with an isotropic voxel resolution of 0.88x0.88x1.2 mm, no slice gap, and FOV = 256x256 mm.
Motion Censoring
To guard against the effects of in-scanner micro-motion on connectivity patterns we implemented motion-censoring, also known as “scrubbing” (Power, et al., 2012; Satterthwaite, et al., 2013). To this end we used FSL's motion outliers tool, which is designed to detect timepoints (i.e. frames) in an fMRI dataset that have been corrupted by motion (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLMotionOutliers). This included (1) motion correction of individual participant’s functional data, (2) calculating framewise displacement (FD) for each timepoint, representing head motion as instantaneous rotational and translational displacements in any direction, (3) thresholding FD at 0.35 (~0.35 mm) to identify timepoints with FD's larger than 0.35, and (4) generating a confound matrix to be used in the subject-level general linear model (GLM), which partials out the effects of these timepoints on the analysis without any adverse effects on the statistics (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLMotionOutliers). More specifically, the GLM fully models all the influence of a corrupted timepoint (i.e. “spike”) with a separate parameter estimate (i.e. beta), which means that the intensities at that timepoint (in any voxel) have no influence on any of the other parameter estimates (i.e. spike regression; Satterthwaite, et al., 2013). Thus, any effect of a corrupted timepoint is effectively removed from estimating connectivity patterns (Satterthwaite, et al., 2013).

Region of Interest Definition
Using cytoarchitectonic probabilistic maps of amygdala subnuclei provided in FSL's Juelich histological atlas (Amunts, et al., 2005), BLA and CMA region of interest (ROI) masks were created in both hemispheres (Figure S2). In line with recent developmental studies (Qin, et al., 2014; Qin, et al., 2012), voxels were included in the ROI masks only if the probability of their assignment to the BLA or CMA was higher than any other nearby structures with greater than 40% likelihood. Each voxel was exclusively assigned to only one region, and overlapping voxels were assigned to the region that had the greatest probability (Qin, et al., 2014; Qin, et al., 2012). These masks were used in subsequent iFC and structural analyses.

Signal From White Matter and Cerebrospinal Fluid
Using FSL’s FAST (FMRIB’s Automated Segmentation Tool), we created masks of the white matter (WM) and cerebrospinal fluid (CSF) for each participant, which were eroded at 80% to prevent partial voluming effects with grey matter. These masks were then used to extract the average time series for WM and CSF, and these time series were used as nuisance regressors in the subject-level GLM analysis. This approach effectively removes physiological noise in RS data and is favored above global signal regression, which has been shown to distort connectivity patterns (Saad, et al., 2012; Weissenbacher, et al., 2009).

Spatial Similarity Analysis
Similar to Qin et al. (2012), spatial correlations were computed between each participant’s BLA and CMA connectivity maps to quantify the overall similarity between the BLA and CMA target networks. This provides important complementary information about the functional differentiation and segregation of BLA and CMA networks. To this end, FSL's cross-correlation tool (i.e., FSLCC) was used, which runs cross-correlations be-
Supplementary Results

BLA and CMA Functional Connectivity

Table 2
Clusters and coordinates of between group differences in BLA iFC.

<table>
<thead>
<tr>
<th>Region</th>
<th>Hemisphere</th>
<th>Voxels</th>
<th>Z-value</th>
<th>MNI coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Right BLA iFC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls &gt; PTSD</td>
<td>R</td>
<td>871</td>
<td>3.22</td>
<td>12 66 8</td>
</tr>
<tr>
<td>Controls &gt; PTSD</td>
<td>L</td>
<td>3.41</td>
<td>-18</td>
<td>40 10</td>
</tr>
<tr>
<td>PTSD &gt; Controls</td>
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<td>973</td>
<td>3.87</td>
<td>-24 -58 42</td>
</tr>
<tr>
<td>Parietal Lobe</td>
<td>L</td>
<td>3.40</td>
<td>-22</td>
<td>-56 52</td>
</tr>
</tbody>
</table>

Note: all Z-values are corrected for multiple comparisons at the cluster-level (Z>2.3; p<0.05)

Table 3
Clusters and coordinates of between group differences in CMA iFC.

<table>
<thead>
<tr>
<th>Region</th>
<th>Hemisphere</th>
<th>Voxels</th>
<th>Z-value</th>
<th>MNI coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Right CMA iFC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls &gt; PTSD</td>
<td>R</td>
<td>1659</td>
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<td>46</td>
<td>-60 18</td>
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<tr>
<td>Parietal Lobe</td>
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<td>-34</td>
<td>-32 22</td>
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<tr>
<td>Occipital Cortex</td>
<td>L</td>
<td>3.12</td>
<td>-26</td>
<td>-60 30</td>
</tr>
</tbody>
</table>

Left CMA iFC

<table>
<thead>
<tr>
<th>Region</th>
<th>Hemisphere</th>
<th>Voxels</th>
<th>Z-value</th>
<th>MNI coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTSD &gt; Controls</td>
<td>L</td>
<td>1128</td>
<td>3.53</td>
<td>-20 -8 -38</td>
</tr>
<tr>
<td>Temporal Pole</td>
<td>L</td>
<td>3.42</td>
<td>-38</td>
<td>10 -44</td>
</tr>
<tr>
<td>Orbitofrontal Cortex</td>
<td>L</td>
<td>3.23</td>
<td>-28</td>
<td>16 -26</td>
</tr>
</tbody>
</table>

Note: all Z-values are corrected for multiple comparisons at the cluster-level (Z>2.3; p<0.05)
Conjunction Analysis

To examine whether PTSD may affect common connectivity patterns of BLA and CMA subregions, we performed exploratory conjunction analyses. Specifically, we sought for areas of convergence between BLA and CMA connectivity by combining the thresholded connectivity maps of these subnuclei across subjects in each hemisphere, and selecting only those voxels that reached corrected significance in BLA as well as CMA connectivity maps. This resulted in connectivity maps common to both BLA and CMA subregions (Figure S3), which were used as region of interest masks during higher-level mixed-effects group analyses, in which we tested whether PTSD may affect connectivity patterns common to both BLA and CMA complexes. Similar to the subregion-specific analyses, age, sex, and IQ (demeaned across groups) were included as covariates of no interest, and resulting statistical maps were corrected for multiple comparisons using cluster-based correction ($p<0.05$, initial cluster forming threshold $Z>2.3$). Our analysis, however, revealed that PTSD does not affect connectivity patterns common to both BLA and CMA subregions.

Figure S3. Common connectivity patterns of BLA and CMA subregions. Sagittal, axial, and coronal views of BLA (blue) and CMA (red) target networks, along with areas that both subregions were functionally connected to (i.e., common connectivity; purple). The common connectivity maps (i.e., purple) were used as region of interest masks during higher-level mixed-effects group analyses, in which we tested whether PTSD may affect connectivity patterns common to both BLA and CMA complexes. Upper panel relates to right and lower panel to left BLA and CMA connectivity patterns. Images are in radiological convention (i.e., right is left and vice versa).