Unresolved-disorganized attachment, psychopathology, and the adolescent brain

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SECTION II:

ATTACHMENT, PSYCHOPATHOLOGY AND NEUROIMAGING

Safety and security don't just happen,
they are the result of collective consensus and public investment.
We owe our children, the most vulnerable citizens in our society,
a life free of violence and fear

Madiba - Nelson Rohlilahla Mandela
(1918, Mvezo – 2013, Johannesburg)

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Unresolved-disorganized attachment associated with smaller hippocampus and increased functional connectivity beyond psychopathology

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ABSTRACT

Loss and abuse in children can lead to unresolved-disorganized attachment (Ud). How this condition relates to brain structure and functional connectivity (FC) is not known. We therefore aimed to investigate gray matter volume (GMV) and resting state functional connectivity (RSFC) correlates of Ud in adolescents. Based on previous neuroimaging studies of trauma effects, we hypothesized that structure of the amygdala and hippocampus and FC of the latter would be linked to Ud. Anatomical and RSFC data were collected from a mixed group of adolescents (*N* =74) with symptoms of posttraumatic stress disorder related to childhood sexual abuse (CSA-related PTSD), anxiety/depressive symptoms and without psychiatric disorder as part of the Emotional Pathways' Imaging Study in Clinical Adolescents (EPISCA). Bilateral volumes of amygdala and hippocampus were measured using FSL, and RSFC of the hippocampus was assessed using seed-based correlation. Ud was measured using the Adult Attachment Interview (AAI). Hierarchical regression and correlation were used to assess the associations between Ud (continuous and categorical), brain structure and FC, adjusting for a general psychopathology factor, puberty stage, gender, age, and IQ. Ud was associated with a smaller left hippocampal volume (R^2=.23) and greater FC between the hippocampus and the middle temporal gyrus and lateral occipital cortex. The association of Ud with specific brain structure and FC across psychopathological classifications shows promise for dimensional complements to the dominant classificatory approach in clinical research and practice.

INTRODUCTION

The loss of an attachment figure or the abuse within an attachment relationship are adverse childhood events that may have lifelong somatic, psychiatric and psychosocial consequences for the individual (Anda et al., 2006; Felitti et al., 1998). From an attachment theory perspective (Bowlby, 1969; 1980; Hesse, 2016), loss and abuse increase the likelihood of unresolved-disorganized attachment (UD): the child may show signs of current mental impact from loss of loved ones or abuse, or the child may apply contradictory approach-avoidance strategies to relationships with parents or other attachment figures. For example, the child may simultaneously display proximity seeking and avoidant behaviors. This UD attachment is considered a (momentary) breakdown of an organized strategy to deal with stressful situations. It results from abuse or another traumatic experience within the attachment relationship, thus confronting the child with a paradox, as the parent is both a source of comfort and fear at the same time for the child (Lyons-Ruth & Jacobvitz, 2016). Not all children are able to resolve these traumatic attachment experiences. Some adolescents show signs of disorientation and disorganization while discussing early traumatic attachment events, indicating that they are still overwhelmed by the trauma or the loss experience (Hesse, 2016; Lyons-Ruth & Jacobvitz, 2016). This UD mental representation may negatively impact current and future attachment relationships and the transition to adult functioning (Hesse, & Main, 2000).

Previous research had indicated that experiences of loss and abuse increase an individual's risk for psychopathology, including posttraumatic stress disorder (PTSD), as well as anxiety or depressive disorders (Cloitre et al. 2009; Gospodarevskaya, 2013; McLaughlin, Sheridan, & Lambert, 2014b). This may be at least partially attributable to the profound adverse effects of early life stress on brain development, particularly the hippocampus, a stress-sensitive brain region that plays a role in the regulation of the hypothalamic–pituitary–adrenal (HPA) axis. Previous studies found evidence for a smaller hippocampal volume in healthy adults with experiences of abuse but not in children (Calem, Bromis, McGuire, Morgan, & Kemptona, 2017; Hart, & Rubia, 2012; Riem, Alink, Out, Van IJzendoorn, & Bakermans-Kranenburg, 2015; Rinne-Albers, Van der Wee, Lamers-Winkelman, & Vermeiren, 2013). A reduced hippocampal volume has also been found in adults with PTSD (Chen, & Etkin, 2013). Studies examining brain structure in patients with anxiety and depressive disorders have shown diverging results, from a larger amygdala volume to a smaller left hippocampal volume (DeBellis et al., 2000; Koolschijn, Van IJzendoorn, Bakermans-Kranenburg, & Crone, 2013; MacMillan et al., 2003; Pechtel, Lyons-Ruth, Anderson, & Teicher, 2014; Schmaal et al., 2016). It is, however, possible that experiences of trauma account for hippocampal abnormalities in patients with PTSD, anxiety, and depressive disorders, since a reduced hippocampal volume has been found in maltreated individuals, regardless of psychopathology. In addition to structural differences, abnormalities in resting state functional connectivity (RSFC) of the hippocampus have been found in individuals with childhood adversity (Philip et al., 2013) and in a variety of neuro-psychiatric disorders known to be related to childhood adversity, such as PTSD (Tursich et al., 2015), depression and anxiety (Veer et al., 2010). More specifically, individuals who have experienced childhood adversity with or without psychopathology show aberrant resting-state connectivity between the amygdala and frontal regions (for a review, see Teicher, & Samson, 2016; Teicher, Samson, Anderson, & Ohashi, 2016). However, there is a lack of research examining the role of attachment in structural and functional brain abnormalities in adolescents with psychopathology, possibly because simultaneous assessment of psychopathology and attachment representations in adolescents is scarce (Van Hoof, van Lang, Speekenbrink, van IJzendoorn, & Vermeiren, 2015).

Attachment is best described as the innate system that motivates humans to develop an affective bond with a protective caregiver as a secure haven and a safe base to explore the environment (Bowlby, 1969). Caregiver protection against dangers and stresses along with stimulation of exploration shape the child's emotion regulation and the ability to build trusting relationships with others (Cassidy, 2016). According to attachment theory, interactions with attachment figures in childhood develop into inner working models of the self and others (Bretherton, & Munholland, 2016). Attachment in adolescents and adults can be assessed with the well-validated Adult Attachment Interview (AAI) (Hesse, 2016; Main et al., 1985), which asks respondents for current mental representations of childhood attachment experiences. In the case of adversity such as loss of an attachment figure or the experience of child abuse, attachment representations may be characterized as UD (Hesse, 2016; Lyons-Ruth, & Jacobvitz, 2016), indicated by incoherent, that is disoriented and disorganized, speech in response to questions about losses or other potentially traumatic events, independent from assessed psychopathology. This UD representation is considered a trans-diagnostic risk factor that may increase vulnerability to a range of psychiatric disorders. Indeed, the authors of a meta-analysis found UD had a prevalence of 43% in combined clinical samples, with elevated rates of unresolved loss and trauma in all clinical groups (Bakermans-Kranenburg, & Van IJzendoorn, 2009).

Some studies point to an association between disorganized attachment and structural brain abnormalities. For example, maltreatment reported in the AAI was associated with smaller hippocampal volume in a study in female adult twin pairs (Riem et al., 2015). Recently, Lyons-Ruth, Pechtel, Yoon, Anderson, and Teicher (2016) showed that both maternal and infant components of disorganized attachment interaction in infancy were associated with increased left amygdala volume later in adulthood in a sample of impoverished, highly stressed families. However, it is yet unknown whether attachment representation as assessed with the 'gold standard' AAI (Hesse, 2016; Main et al., 1985) is associated with structural brain abnormalities. Moreover, whereas there is sparse literature on the relationship between attachment and brain morphology, studies on attachment representation and functional connectivity in the brain are lacking. Examining how UD attachment relates to brain structure and functional connectivity will extend previous neuroimaging research on childhood trauma, as previous studies assessed trauma retrospectively and have not examined whether or not it matters if the trauma has been resolved. UD attachment represents a *current* state of mind with respect to childhood attachment experiences. It is yet unknown how this current state relates to brain measures.

Although there is evidence that UD attachment increases vulnerability to psychopathology in general, it is unknown how unresolved status relates to the abnormalities in brain structure and function that are commonly found in patients with psychopathology (Caspi et al., 2014; Lahey et al., 2017). In the current study, we therefore examined whether UD attachment is related to brain abnormalities across multiple psychiatric diagnoses. Thus, we applied a dimensional approach to examine grey matter and resting-state abnormalities related to UD attachment across different psychopathological conditions. Although traditionally psychiatric disorders have been viewed as categorical psychopathological conditions, recent research shows accumulating evidence for a dimensional approach of psychopathology and points to overarching features and trans-diagnostic factors. This dimensional approach to the structure of psychopathology may explain high levels of comorbidity among mental disorders. However, clinical neuroscience has not kept pace with these advances (Zald & Lahey, 2017). Neuroimaging studies examining biomarkers for psychopathological conditions point to similar structural and functional brain abnormalities across psychopathological conditions (Zald & Lahey, 2017). These shared brain abnormalities may be explained by high levels of comorbidity or shared trans-diagnostic risk factors, such as UD attachment.

Therefore, the aim of the present study was to investigate whether UD attachment representation is associated with gray matter volume (GMV) of the hippocampus and amygdala in a sample of adolescents, after adjusting for psychiatric symptomatology. We chose the hippocampus and amygdala as regions of interest, based on previous studies showing abnormalities in these regions in individuals with experiences of childhood trauma. In addition, we examined whether brain regions that show structural alterations related to UD attachment are also associated with different functional resting state connectivity. In sum, we examined the neural correlates of unresolved for loss or trauma as assessed with the AAI (Main et al., 1985). Our hypothesis is that UD attachment would be correlated with a smaller hippocampal volume and a larger amygdala (Brenning, & Braet, 2013; Brown, & Morey, 2012) and that brain structures associated with UD attachment would also show alterations in functional connectivity.

METHOD

Participants and procedure

Sample

Participants. The current study involved 74 participants from the Emotional Pathways' Imaging Study in Clinical Adolescents (EPISCA) (Van Hoof, et al., 2015; *N*=77) were involved in the current study. They were recruited according to specified inclusion and exclusion criteria (Van den Bulk et al., 2013; Van Hoof et al., 2015; see supplemental material) and available coded AAIs (Main et al., 1985). Drop-out was due to anomalous magnetic resonance imaging (MRI) findings (*n*=2), technical scanning problems or poor imaging data quality (*n*=2). Within this group, there were 21 adolescents with PTSD related to childhood sexual abuse (CSA), 28 adolescents with anxiety and/or depressive disorders (DEP) and 25 non-clinical adolescents (CNTR). All adolescents with experiences of CSA had PTSD. Some adolescents in the DEP and CNTR group were exposed to other types of trauma (see supplemental material) but not to CSA. Inclusion criteria for the CSA group were having experienced sexual abuse during their lifetime more than once by one or more perpetrators in- or outside the family, and being referred for treatment. See Van Hoof and colleagues (2015) for a detailed description. The sample was originally recruited based on whether they had experienced CSA, had an anxiety and/or depressive disorder or had no clinical symptoms in order to be able to compare groups cross-sectionally (see Van Hoof et al., 2015). In the current study, the CSA, DEP, and CNTR groups were analyzed together as the aim was to examine whether UD attachment is related to brain abnormalities across multiple psychiatric diagnoses.

Power analysis using G* power (linear multiple regression) showed that the power needed to examine effects of UD and the general psychopathology factor (GPF) on brain structure was met with an alpha value set to .05 and a power of .80, with an expected medium effect size *f* = 0.15 (Calem et al., 2017), and two predictors (UD and GPF), with a required sample size of 68.

The study sample comprised 63 females (85.1%), with 18 in the CSA group, 24 in the DEP group, and 21 in the CNTR group. Participants' mean age was 15.42 years (SD 1.67, range 12-20), and they had a total mean IQ of 103.28 (SD 8.89, range 81-119). Regarding cultural background, 1.4% of participants were Asian (CSA *n*=1), 93.2% were Caucasian (CSA *n*=20, DEP *n*=25, CNTR *n*=24), 1.4% were Surinamese (DEP *n*=1), 2.7% were Latin-American (DEP *n*=2). Four adolescents (5.4%; CSA *n*=2, DEP *n*=2) reported stable selective serotonine reuptake inhibitor use (*n*=3 on fluoxetine, *n*=1 on sertraline). Puberty stage was assessed using the Pubertal Development Scale (PDS; Petersen, Crockett, Richards, & Boxer, 1988) according tot he following categories: prepubertal (CSA *n* =1), midpubertal (CNTR *n* =5), late pubertal (CSA *n* =7, DEP *n* =11, CNTR *n*=12), postpubertal (CSA *n* =10, DEP *n* =9, CNTR *n* =5). Information about pubertal status was missing for 10 participants; for these participants, pubertal status was imputed using gender and age. Attachment and clinical characteristics of the original larger total sample (*N*=77) not using imaging data, have been reported separately (Van Hoof et al., 2015).

Written informed assent and consent was obtained from all adolescents and their parents. Participants received a financial compensation including travel expenses. The medical ethics committee of the Leiden University Medical Centre approved this study. After adolescents and their parents had given assent and consent to participate in the EPISCA study, they filled out questionnaires (usually at home), and were tested for IQ and interviewed for classification of any disorder according to the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-IV*) and attachment representation at the clinic in separate appointments. Scanning was usually performed separate from the aforementioned appointments, depending on availability of the scanner.

Measures

Attachment

Adult Attachment Interview. The AAI (Main et al., 1985; see Supplemental Material) is a clinician-administered semi-structured interview, validated for adolescents, that takes approximately 1 hr to administer. The AAI asks interviewees how they think about their relationship with parents or other primary caregivers in their youth, how these experiences have influenced them, how their actual relationship with parents or other primary caregivers is, and whether there were any experiences of illness, separation, fear, trauma or loss. Interviewees are asked to give specific examples supporting each evaluation. The coherence of the narrative rather than its autobiographical content is of most importance.

After transcription and coding of the AAI according to the manual (Hesse, 2016) by a certified coder, an attachment representation classification can be given. In organized attachment representations there is one coherent mental strategy with regard to attachment figures, either secure-autonomous or insecure. In UD attachment representations, different mental strategies with regard to attachment figures are used simultaneously or sequentially, often contradicting one another, which becomes apparent when coding the narrative. The AAI includes a dimensional subscale entitled Unresolved for Loss or Trauma; AAI narratives are assigned scores on this dimension between 1 and 9, with score of 9 indicating verbal behavior with highly incoherent speech characteristics in the narrative around loss or trauma experiences. A scale score for Unresolved Loss or Trauma of 5.5 or above also renders an individual UD (see Supplemental Material).

General Psychopathology Factor

To control for the effects of psychopathology, we decided to use the GPF. The GPF represents the lesser-to-greater severity of psychopathology associated with negative emotionality (Tackett et al., 2013), compromised brain integrity (Caspi et al., 2014), lower IQ, higher levels of negative affectivity, and lower levels of effortful control shown in 1,954 children between 6 and 8 years of age from a birth cohort (Jaddoe et al., 2012; Neumann et al., 2016). The GPF shows a significant Single Nucleotide Polymorphism (SNP) heritability of 38% (SE=0.16), p=.008. The use of the GPF has also been shown to be valid in girls (Lahey et al., 2015) and in young adolescents (Patalay et al., 2015). In our sample, the GPF was estimated using parent and self-report measurements for behavioral and emotional problems in children and adolescents: the Youth Self Report (YSR; Achenbach, 1991a; Verhulst, Ende, & van der Koot, 1997), Child Behavior CheckList (CBCL; Achenbach, 1991b; Verhulst, Ende, & van der Koot, 1996), Revised Child Anxiety and Depression Scale (RCADS; Chorpita, Yim, Moffitt, Umemoto, & Francis, 2000; Oldehinkel, 2000), Trauma Symptom Checklist for Children (TSCC; Briere, 1996), Children's Depression Inventory (CDI; Kovačs, 1992), and Adolescent Dissociative Experiences Scale (A-DES; Armstrong, Putnam, Carlson, Libero, & Smith, 1997). Principal Component Analysis was performed using these scales and appropriate subscales, and one component explaining 61.6% was extracted, all loadings > .56, see Supplementary Table S3). Factor scores were calculated in order to estimate the GPF (Franke, 2016; Lahey et al., 2012; Lahey, Zald, et al., 2017; Lahey, Krueger, Rathouz, Waldman, & Zald, 2017). See Supplemental Material for a detailed description of the questionnaires used to estimate the GPF.

Image data acquisition

Images were acquired on a Philips 3T MRI system (Philips Healthcare, Best, the Netherlands), equipped with a SENSE-8 head coil. Scanning took place at the Leiden University Medical Centre. Prior to scanning, all participants were prepared for scanning by lying in a dummy scanner and hearing scanner sounds. For each participant, a sagittal 3-dimensional gradient-echo T1-weighted image was acquired (repetition time=9.8 ms; echo time=4.6 ms; flip angle=8°; 140 sagittal slices; no slice gap; field of view = 256 × 256 mm; 1.17 × 1.17 × 1.2 mm voxels; duration= 4:56 min) as part of a larger, fixed imaging protocol. Resting-state functional MRI (fMRI) data were acquired, using T2*-weighted gradient-echo echo-planar imaging (160 whole-brain volumes; repetition time 2,200 ms; echo time 30 ms; flip angle 80°; 38 transverse slices; no slice gap; field of view 220 mm; in-plane voxel size 2.75 x 2.75 mm; slice thickness 2.72 mm; total duration of the resting-state run = 6 min). Participants were instructed to lie still with their eyes closed and not to fall asleep.

Data analysis

Hippocampal and amygdala volumes. Volumes of the left and right hippocampus and amygdala were assessed using FMRIB's Integrated Registration and Segmentation Tool (FIRST; Patenaude, Smith, Kennedy, & Jenkinson, 2011), part of FSL FMRIB's Software Library, http://www.FMRIb.ox.ac.uk/fsl (Smith et al., 2004). Hippocampal volumes were extracted after affine registration to standard space and subcortical structure segmentation. Registrations and segmentations were visually inspected, and no errors were observed. After hippocampal volume extraction, fslstats was used to assess volumes of the left and right hippocampus and amygdala. Brain tissue volume, normalized for participant head size, was estimated with SIENAX (Smith, De Stefano, Jenkinson, & Matthews, 2001; Smith, 2002). Brain and skull images were extracted from the single whole-head input data (Jenkinson, Bannister, Brady, & Smith, 2002). The brain image was then affine registered to MNI152 space (Jenkinson et al., 2002), after which tissue-type segmentation with partial volume estimation was carried out in order to calculate total brain volume, including separate estimates of volumes of gray matter, white matter, peripheral gray matter and ventricular CSF (Zhang, Brady, & Smith, 2001). Volumes of the left and right hippocampus and amygdala and total brain volume (mm^3) were exported to SPSS.

First, four hierarchical regression analyses with left and right hippocampal volume and left and right amygdala volume were performed with the GPF, sex, composite score age/pubertal status (see Supplemental Material), total IQ score, and whole brain volume in the Step 1 and unresolved loss or trauma (categorical UD vs. non-UD and unresolved continuous scale in two separate models) in Step 2. All participants were included in the UD versus non UD comparison, and analyses were performed with the clinical groups combined, as the aim of the study was to apply a dimensional approach. In addition to age, pubertal status was also included because variance in pubertal status may be related to different brain structures than variance in age. A composite score for age and pubertal status was calculated in order to control for multicollinearity (Giedd et al., 2006). Statistics indicated no multicollinearity, largest Variance Inflation Factor (VIF) ≤ 1.20, tolerance > .83). The four hierarchical regression analyses were repeated with the GPF as an additional covariate in the first step. Vertex analysis was performed using first_utils (Patenaude et al., 2011) in order to localize and visualize effects of Unresolved status. Exploratory whole brain VBM analyses were performed.

Functional connectivity. Contrasts of interest were the parameter estimates corresponding to the regressor of the region that was significantly related to unresolved loss and trauma (a subregion of the left hippocampus; see Figure 2), which represents functional connectivity with that region. Thus, the left hippocampus was used as the seed region. After transforming the mask to native space, the mean time series for each participant was extracted from the left hippocampus using *fslmeants*. The time series was then used as a regressor in the model. In addition, CSF, white matter and the global signal (see Supplemental Material) were added as regressors to the model in order to reduce the influence of artifacts caused by physiological signal sources on the results (Fox, & Raichle, 2007). The temporal derivative of each regressor was added to the model, which resulted in eight regressors in each model. Motion parameters were also added to the model. First-level analyses were performed in native space. These first-level contrast images and the corresponding variance images were transformed to standard space and submitted to second-level mixed-effects group whole brain analyses. The positive and negative correlation between hippocampal connectivity and unresolved loss and trauma score were assessed as were the contrasts UD greater than nonUD and UD smaller than nonUD. Thus, we contrasted UD with non-UD and applied a dimensional analysis of UD. We included the GPF, composite score age and pubertal status, sex, and IQ as confound regressors in the model. The statistical images were corrected for multiple comparisons at the cluster level in FSL, with a cluster-forming threshold of $Z > 2.3$ and a cluster-corrected significance of $p < .050$ (Worsley, 2001). This threshold was chosen to balance Type I and Type II error, as has been recommended (Hopfinger, 2017; Slotnick, 2017). Harvard-Oxford cortical structural atlas was used to localize hippocampal connectivity.

RESULTS

Clinical sample characteristics

See Table 1 for the clinical sample characteristics. Based on the AAI (Cassidy, 2016) 36.5% of the adolescents were classified as secure (CNTR *n*=13, DEP *n*=11, CSA *n*=3), 41.9% as dismissive (CNTR *n*=11, DEP *n*=11, CSA *n*=9), and 21.6% as UD (CNTR *n*=1, DEP *n*=6, CSA *n*=9). Unresolved-disorganized attachment was found in 16 (21.6%) participants. Of these unresolved participants, six adolescents had anxiety and/or depressive disorders, and nine had CSA-related PTSD. See Supplementary Table S1 for psychopathology scores for the separate groups (CSA-PTSD, internalizing, control and U vs. nonU).

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Table 1. *Psychiatric Symptom Scores for the Whole Sample, Measured with the YSR, CBCL, RCADS, TSSC, CDI, and A-DES.*

Clinical characteristic	M	SD	Range
Depression	12.84	9.17	0-40
Posttraumatic stress	34.13	22.72	0-98
Anxiety	25.88	14.96	0-70
Dissociation	1.44	1.42	0-6.37
Internalizing youth report	18.78	11.13	0-44
Internalizing parent report	13.60	9.68	0-42
Unresolved attachment	2.40	1.18	1-8

Volumetric measurement of amygdala and hippocampus

Hierarchical regression analyses showed a significant effect of UD versus non UD on left hippocampal volume ($F_{(5,68)} = 3.94$, $p = .003$, $R^2 = .17$), but not on right hippocampal volume or on amygdala volume (left or right; see Supplementary Table S2). Hierarchical regression analyses were repeated with the GPF as an additional covariate. Again, there was a significant effect of the categorical UD versus non UD on left hippocampal volume beyond psychopathology ($F_{(6,67)} = 3.37$, $p = .014$, $R^2 = .23$). Participants who were classified as UD showed a smaller left hippocampal volume (M = 3,574.33, SD = 510.99 for UD; M = 3,921.81, SD = 344.29 for non-UD). The effect of UD remained significant after excluding one control participant with UD status. Hierarchical regression analysis with the continuous variable unresolved for loss or trauma (U) as predictor did not show a significant effect of U on bilateral hippocampal volumes beyond psychopathology (see Table 2). No effect was found of UD versus non UD on right hippocampal volume (see Table 2) or in the hierarchical regression analyses with the amygdala as the dependent variable (see Table 3). Vertex analysis to localize and visualize the effect of UD in specific subfields of the hippocampus was marginally significant, $p < .100$, corrected for multiple comparisons. The hippocampal region of interest is shown in Figure 1. Exploratory whole brain analyses yielded no results.

Table 2. *Results of Hierarchical Regression Analyses with Hippocampal Volume (L/R) as the Dependent Variable, Adjusting for Sex, Age/Pubertal Status, Total IQ Score, General Psychopathology Factor (GPF) in Step 1 and Unresolved Loss or Trauma Status in Step 2.*

	Left hippocampus					Right hippocampus				
	B	SE	ß	*p*	delta R^2	B	SE	ß	*p*	delta
Step 1					.16*					.14
Sex	-195.73	132.21	-.17	.143		-277.48	140.85	-.23	.053	
Age-Puberty	-4.90	43.83	-.01	.911		9.87	46.70	.03	.833	
WBV	0.00	0.01	.00	.970		0.00	0.00	.10	.407	
TIQ	16.38	5.15	.36	.002		12.27	5.49	.25	.029	
GPF	19.99	47.76	.05	.677		-7.20	50.88	-.02	.888	
Step 2					.03					.03
Ud versus non Ud	-282.99	111.64	-.29	.014		-197.54	122.45	-.19	.111	
U continuous	-262.78	158.58	-.20	.102		-274.67	169.08	-.20	.109	

Note. Age-puberty is composite score of age and puberty status; WBV =Whole Brain Volume ; TIQ = Total Intelligence Quotient ; Ud = unresolved–disorganized attachment (categorical); U = unresolved loss or trauma (continuous) .

Table 3. *Results of Hierarchical Regression Analyses with Amygdala Volume (L/R) as the Dependent Variable, Adjusting for Sex, Age/Pubertal status, Total IQ Score, and General Psychopathology Factor (GPF) in Step 1 and Unresolved Loss or Trauma*

Status in Step 2.

	Left amygdala					Right amygdala				
	B	SE	ß	*p*	delta R^2	B	SE	ß	*p*	delta R^2
Step 1					.07					.03
Sex	-131.26	70.93	-.23	.069		95.77	86.26	-.14	.271	
Age-Puberty	27.32	23.52	.15	.249		14.67	28.60	.07	.610	
WBV	0.00	.00	.10	.412		0.00	0.00	-.80	.513	
TIQ	2.06	2.76	.09	.458		0.14	3.36	.01	.966	
GPF	6.83	25.62	.03	.790		11.87	31.16	.05	.704	
Step 2										
Ud vs. non Ud	79.68	61.94	-.16	.203	.02	-109.89	75.06	-.18	.148	.03
U continuous	-6.78	86.80	-.01	.938	.00	39.08	105.46	-.05	.712	.00

Note. Age-puberty is composite score of age and puberty status; WBV = Whole Brain Volume ; TIQ = Total Intelligence Quotient ; Ud = unresolved–disorganized attachment (categorical); U = unresolved loss or trauma (continuous) .

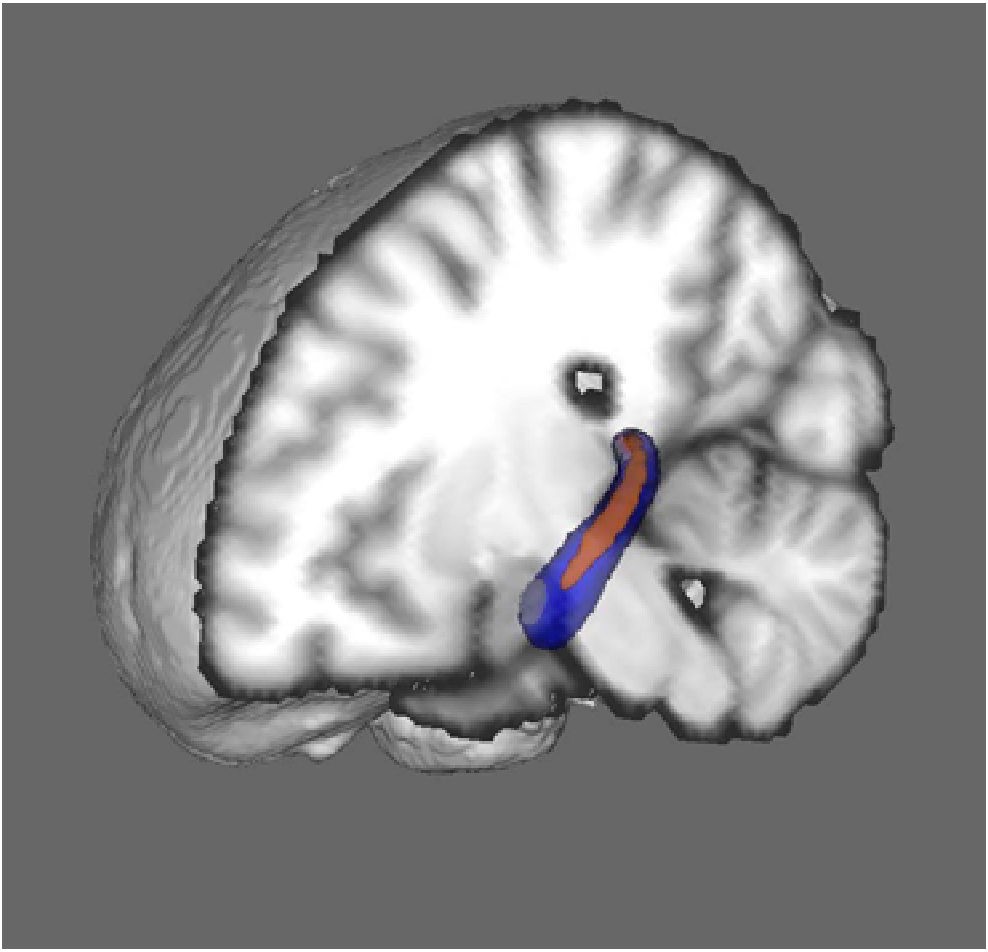

Figure 1. Red shading shows reduced hippocampal volume in adolescents with an unresolved-disorganized (UD) status compared to adolescents without a UD status, $p < .100$ (corrected for multiple comparisons). Blue shading shows study-specific mask of the left hippocampus.

Resting state functional connectivity

Analyses of RSFC showed that Unresolved loss or trauma was positively related to connectivity between the left hippocampus and the right middle temporal gyrus (MTG) and the lateral occipital cortex (LOC), cluster size = 654 voxels; peak Z = 3.55; MNI coordinates x,y,z (mm) = 40, -60, 10 (see Figure 2). In addition to the analysis with the dimensional measure of UD, we contrasted UD versus non UD, but there was no significant difference in hippocampal connectivity between the UD versus the non UD group.

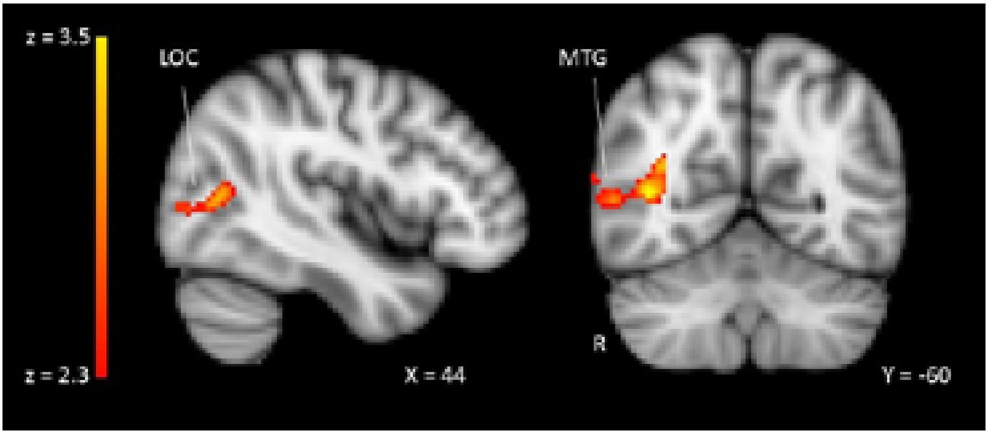

Figure 2. Results of the resting-state functional connectivity analysis. Unresolved loss and trauma is positively associated with connectivity between the left hippocampus and the middle temporal gyrus (MTG) and the lateral occipital cortex (LOC). Cluster thresholded $Z > 2.3$, $p < 0.050$.

DISCUSSION

The aim of this study was to investigate whether UD attachment representation as assessed with the AAI was associated with different volumes of hippocampus and amygdala as well as with related differential connectivity in hippocampus or amygdala-based RSFC networks in adolescents with CSA-related PTSD, anxiety and/or depressive disorders and those without psychiatric symptoms. As recent research shows accumulating evidence for a dimensional approach of psychopathology and points to overarching features and trans-diagnostic factors, we applied a dimensional approach to examine grey matter and resting-state abnormalities related to UD attachment across different psychopathological conditions. Unresolved versus resolved group status was associated with a significantly smaller left hippocampal volume after adjusting for general psychopathology, puberty status, age, gender, and IQ. In addition, there was a positive correlation between UD attachment score and left hippocampal functional connectivity with the right MTG and LOC. No associations were found between UD attachment and right hippocampus or amygdala volumes.

Our findings are consistent with research showing that UD attachment is a trans-diagnostic risk factor that increases vulnerability to psychopathology in general. Moreover, these findings indicate that hippocampal abnormalities previously found in patients with PTSD, depression or anxiety disorders are not a specific biomarker for individual mental disorders, but instead are common to several disorders, and could be related to etiological factors rooted in childhood attachment experiences. The hippocampus is one of the most stress-sensitive structures in the brain, as it modulates the HPA axis responsiveness to stress (Bernard, Lind, & Dozier, 2014). Early life stress such as child abuse and neglect may reduce the number of hippocampal glucocorticoid receptors, prevent neurogenesis, and distort synaptic pruning (Sapolsky, Krey, & McEwan, 1985; Sapolsky, Uno, Rebert, & Finch,1990). In response to stress, the hypothalamus releases corticotrophin-releasing hormone and arginine vasopressin. This leads to the secretion of adrenocorticotrophic hormone and increased cortisol release. When cortisol binds to glucocorticoid receptors in the hippocampus, hypothalamus, and the pituitary, inhibitory feedback is given which returns the system to homeostasis (Koss, & Gunnar, 2018). Damage to the hippocampus results in reduced glucocorticoid-mediated feedback control of the HPA axis, leading to hyper- or hypo-responsiveness to mild stressors (McCrory, De Brito, & Viding, 2011), which in turn may explain poor emotion regulation and increased risk for psychopathology in individuals with unresolved trauma.

We found smaller left hippocampal volume in the UD versus organized adolescent group. This finding is in line with previous findings showing a smaller left hippocampal volume in adults with experiences of maltreatment (Riem et al., 2015). Maltreatment-related PTSD in children, however, was not related to hippocampal volume in a meta-analytic study (Woon, & Hedges, 2008); additionally, a study on the neurobiological effects of poor caregiving in orphanage reared children did not demonstrate a smaller hippocampus (Tottenham et al., 2010). One explanation could be that the sexual and physical abuse reported in the current study took place from early childhood to adolescence, a developmental period that is most sensitive to negative effects of maltreatment (Riem et al., 2015). Thus, the timing of the abuse may matter. Also, the reported abuse was often severe, cumulative and protracted, and the treatment gap between the abuse and start of treatment was sometimes rather large (Van Hoof et al., 2015), all of which may have negatively impacted the hippocampal volume due to severe and prolonged stress. Moreover, neuro-anatomic findings according to age in adolescents may already more closely resemble those in adults than in children. Another plausible explanation may be that UD attachment indeed constitutes a different concept than PTSD or maltreatment and shows different findings in relation to the brain when general psychopathology has been controlled for.

In addition, we found that UD attachment was related to the left hippocampus functional connectivity with the MTG and the LOC. In a meta-analysis Sabatinelli and colleagues (Sabatinelli et al., 2011) found activation in both regions related to processing of emotional information. The LOC has been shown to be implicated in higher level visual processing, including emotional scene perception, whereas the MTG seems to be associated with the processing of emotional faces, including faces provoking social aversion (Krause et al., 2016). However, enhanced connectivity between the hippocampus, MTG and LOC was found during rest, which is surprising since the MTG and LOC are not part of the limbic or default mode network. Thus, our finding indicates that UD attachment is related to atypical hippocampal limbic or default mode network connectivity. Future studies should investigate whether neural processing of emotions in individuals with UD attachment is due to their Unresolved status or psychopathology, as altered MTG and LOC activity may also be associated with atypical processing of emotional stimuli of various kinds. Also, individuals with UD attachment may be more vulnerable to associate negative emotional stimuli with their current mental representation of traumatic sexual and/or physical experiences or past losses. The smaller hippocampal volume associated with unresolved loss or trauma may indicate a less effective HPA-axis feedback loop (Gupta, & Morley, 2014) leading to a lowered threshold for experiencing stress through perceptions or memories of loss or trauma.

Contrary to our hypothesis, we did not find an association between UD attachment and amygdala volumes. Our adolescent sample showed left hippocampal reduction but no (left) amygdala enlargement, as would have been in line with what was reported by Lyons-Ruth and colleagues (Lyons-Ruth et al., 2016), who found an association with both maternal and infant disorganization (but not child abuse per se) with larger left amygdala volume in adolescence in a sample of impoverished, highly stressed families. One explanation for the absence of the relation between unresolved status and amygdala volume in the current study is that acute threat and anxiety rather than childhood trauma could be related to amygdala enlargement. This is consistent with neuroimaging studies on affective disorders (Rinne-Albers et al., 2013; Van den Bulk, 2015) and suggested by normal development of hippocampus and amygdala (Tottenham, & Sheridan, 2010).

A previous study that used the same sample but did not include the AAI showed that abnormal amygdalar connectivity related to diminished grey matter of the basolateral and centrolateral subnuclei in the amygdala was associated with psychopathology (Aghajani et al., 2016). In contrast, the current study removed variance associated with psychopathology; therefore, it makes sense that amygdala abnormalities were not detected. The unique contribution of UD attachment on top of this psychopathology seems only related to hippocampal volume and hippocampal functional connectivity with the MTG and LOC which are involved in visual processing.

To the best of our knowledge this is the first study linking adolescent attachment status to amygdala and hippocampal volumes and GMV in the adolescent brains of both clinical and non-clinical individuals. There are, however, some limitations to consider. The generalizability of results may be limited due to the fairly small sample size and the restricted ranges of age, IQ, gender, and ethnicity. Also, this is a cross-sectional study, so reversed causality can easily shape the interpretation of results and definitive conclusions about cause and effects cannot be drawn. Finally, to be rendered UD on the AAI one must have experienced (interpersonal) trauma or loss that is volunteered in responding to some loss- and trauma-related questions on the AAI. Without such a trigger for narrative incoherence in the speech around loss or trauma, it is only possible to rate the individual on the continuous or categorical UD variable as showing the absence of unresolved status.

In conclusion, our study suggests that across diagnoses, UD attachment is associated with structural and functional connectivity abnormalities of the hippocampus, a brain structure involved with regulation of the HPA axis, memory consolidation, and emotion regulation.

DECLARATION OF CONFLICTING INTERESTS
The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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SUPPLEMENTAL MATERIAL CHAPTER 4 (as published online)

<u>METHODS</u>

<u>In- and exclusion criteria EPISCA</u>

The adolescents were part of the EPISCA study (Emotional Pathways' Imaging Study in Clinical Adolescents), a longitudinal study in which adolescents were followed over a six-month period. The adolescents with and without clinical symptoms underwent a diagnostic assessment and an MRI scanning protocol at three points in time (at baseline, 3 months, 6 months)(Van den Bulk et al., 2013). AAI (Main et al., 1985) and clinical characteristics of the group and neuroimaging data were reported previously (Van Hoof et al., 2015; 2017).

Related to the neuroimaging protocol all participants met the following inclusion criteria: aged between 12 and 20 years, estimated full scale IQ $\geq$ 80 as measured by Dutch versions of the Wechsler Intelligence Scales for Children (WISC-III; Wechsler, 1991) or Adults (WAIS-III; Wechsler, 1997), being right-handed, normal or corrected-to-normal vision, sufficient understanding of the Dutch language, no history of neurological impairments and no contraindications for MRI testing (e.g. braces, metal implants, lead tattoos, irremovable piercings, claustrophobia or possible pregnancy). The adolescents with childhood sexual abuse (CSA) were recruited at two psychotrauma centres of child and adolescent psychiatric institutes in the Leiden region in the Netherlands. Inclusion for CSA was having experienced sexual abuse during their lifetime more than once by one or more perpetrators in- or outside the family, and being referred for treatment at the psychotrauma centre. The inclusion criteria for adolescents with anxiety and/or depressive disorders were: being referred for outpatient treatment, having a clinical diagnosis of DSM-IV depressive and/or anxiety disorders (Silverman et al., 2001) and no history of CSA (see Aghajani et al. ,2013; Pannekoek et al., 2014a; 2014b). Exclusion criteria for both clinical groups were: (1) a primary DSM-IV diagnosis of Attention Deficit and Hyperactivity Disorder, Oppositional Defiant Disorder, Conduct Disorder, Pervasive Developmental Disorders, Tourette's syndrome, Obsessive-Compulsive Disorder, bipolar disorder, and psychotic disorders; (2) amphetamine medication on the day of scanning or current use of psychotropic medication other than stable use of SSRI's; and (3) current substance abuse. The non-clinical adolescents were recruited through local advertisement, with the following inclusion criteria: no clinical scores on validated mood and behavioral questionnaires or past or current DSM-IV classification, no history of traumatic experiences and no current psychotherapeutic intervention of any kind.

To objectify any abuse or neglect as well as risk for functional impairment and morbidity (Karam et al., 2014) we verified police reports, involvement of child welfare, and family custody or other child protection measures as to have an estimate of the severity and impact of problems. Most adolescents with CSA (87%) reported during the AAI serious and/or longstanding physical sexual contact including repeated or group rape, in 63.6% by a person other than an attachment figure. In addition, 36.4% of the CSA group also experienced physical abuse, 22.7% by a person other than an attachment figure, 9.1% by an attachment figure, in one case by both. Sexual abuse was reported to the police in 60.9%, child welfare was involved in 56.5% of the cases, while 17.4% had a child protection measure (family custody). None of the participating non-clinical adolescents and those with anxiety and/or depressive disorders had experienced CSA, but they did mention physical and emotional abuse, bullying, and other incidents. Non-clinical adolescents had not been involved with police, child welfare or child protection, while 23% of the adolescents with anxiety and/or depressive disorders had child welfare involvement.

From the original sample of 82 adolescents, three participants were excluded due to technical problems, i.e. failed voice and video recording (one adolescent with CSA), unintelligible recording (one non-clinical adolescent), incorrect interview technique (one non-clinical adolescent). Two participants (one non-clinical adolescent and one adolescent with anxiety/depressive disorder) were excluded because they refused the AAI because of the interview itself. Of the *N*=77 in the remaining sample, 86% were girls. All CSA adolescents fulfilled the DSM-IV criteria for PTSD, according to the ADIS (Silverman et al. 2001), however one adolescent missed a point on the interference score to fully qualify for PTSD. SSRI's were used by four of the adolescents with CSA and two of those with anxiety and/or depressive disorder.

YSR: Youth Self-Report (Achenbach, 1991a) and CBCL: Child Behaviour Checklist (Achenbach, 1991b), with Dutch translations by Verhulst and colleagues (Verhulst et al., 1996; 1997). The YSR and CBCL are self-report questionnaires using a 3-point scale to assess social-emotional and behavioural problems in adolescents. The CBCL is the questionnaire for parents, the YSR for adolescents 11 years and older. There are 9 subscales and 3 main scales (total score, externalizing problemscore and internalizing problemscore). In this study, we used the internalizing problemscores of the YSR and CBCL.

ADIS: The Anxiety Disorders Interview Schedule Child and Parent Versions (ADIS C/P; Silverman et al., 2001) are semi structured interviews designed specifically for DSM-IV classification of anxiety and other related disorders such as depression and PTSD in children and adolescents. Strong test-retest reliability was shown for combined and individual ADIS-C/P diagnoses. Intra-class correlations were excellent. Interrater reliability between child and parent versions of the ADIS was reported to be excellent. In this study, the ADIS was applied to all participants by certified trained clinicians and researchers.

TSCC: The Trauma Symptom Checklist for Children (TSCC)(Briere, 1996) is a 54-item self-report for children and adolescents aged 8-17, which measures trauma-related symptoms. In the present study, only the TSCC total score was used as subscales overlapped significantly, with a Cronbach's alpha coefficient of .96.

A-DES: The Adolescent Dissociative Experiences Scale (Armstrong et al., 1997) is a self-report for adolescents aged 11-18 measuring possible dissociation. The A-DES has good reliability and validity. In this study, the mean total score on the A-DES was used as a measure of dissociation, which had a Cronbach's alpha coefficient of .95.

CDI: The Children's Depression Inventory (Kovačs, 1992) is a 27-item, self-rated, depression symptoms-oriented scale suitable for youths aged 7 to 17. The CDI has good psychometric properties of validity and reliability (Cronbach's alpha .71 to .86)(Timbremont et al., 2004) though discriminant validity has been subject to discussion. In this study, the total CDI score had a Cronbach's alpha coefficient of .93.

RCADS: The Revised Child Anxiety and Depression Scale (Chorpita et al., 2000; Oldehinkel, 2000) is a self-rated, anxiety and depressive symptoms-oriented 47-item-scale for children aged 6 to 18. Items are scored based on a four-point scale and grouped as depressive disorder, generalized anxiety disorder, social phobia, anxiety disorder NAO and obsessive-compulsive disorder. Chorpita et al. (2000) reported evidence for validity and reliability of the RCADS in clinical and healthy control adolescents. In this study, the total score of the RCADS was used as a measure for severity of experienced symptomatology (Cronbach's $\alpha = .95$). Besides, the depression scale (Cronbach's $\alpha = .89$) and the cumulative anxiety scales (Cronbach's $\alpha = .94$) were used.

AAI: the Adult Attachment Interview (Main et al., 1985) is coded according to the DEFU system (Hesse, 2016): dismissive (Ds), preoccupied (E), secure-autonomous (F), unresolved-disorganized (Ud). Ds, E and F classifications are organized forms of attachment, while Ud represents disorganized forms of attachment. In organized attachment representations there is one coherent mental strategy with regard to attachment figures, either secure-autonomous (F) or insecure (Ds or E). In disorganized attachment-representation different mental strategies with regard to attachment figures are used simultaneously or sequentially, often contradictory. A high to moderate coherence of the narrative is seen in secure-autonomous (F) attachment interviews in which the interviewee can give ample evidence for general evaluative statements made regarding attachment relationships and attachment experiences whether good or bad. In case of unresolved loss or trauma, the attachment representation is labeled unresolved-disorganized (Ud). This classification can be given in addition to a Ds, E or F classification. A fifth category, cannot classify (CC), is used when the interviewee presents contrasting attachment strategies for attachment figures in the course of the interview resulting in very low coherence of narrative. In most studies U and CC are combined in one category, Unresolved-disorganized. Coherence of mind and unresolved for loss or trauma (Ulosstrauma) are two dimensional scales of the AAI which are assigned scores rated between 1-9. Lowest score for Coherence means there is little or no coherence of mind, highest score for Ulosstrauma means there is high impact of loss or trauma.

The AAI has been administered to more than 10,000 respondents since its development (Bakermans-Kranenburg, & Van IJzendoorn, 2009). The AAI is found to have remarkably good test-retest, discriminant reliability as well as predictive validity. In this study, the AAI was administered by MJvH and CIG, verbatim transcribed according to protocol, and coded by GK (trained by Diane and Dave Pederson), and SdH (trained by Diane and Dave Pederson, and June Sroufe). Both reached intercoder reliability standards in the AAI classification system. Ten cases were also coded by MJBK. Interrater agreement in this sample was 80% for F-nonF, 90% for Ud-nonUd and 70% for four-way classification (DEFU). Kappa's for coding F-nonF (.59) and Ud-nonUd (.62) were both statistically significant and reasonable to satisfactory (see also Van Hoof et al., 2015).

WISC-III-NL and WAIS-III: Short versions of the Wechsler Intelligence Scale for Dutch Children aged 6-16 years, WISC-III-NL (Wechsler, 1991; Crawford et al., 1996) and adolescents aged 16 and above and adults, the Wechsler Adult Intelligence Scale, WAIS-III, (Wechsler, 1997) were used. They consisted of six subtests: picture completion, similarities, picture arrangement, arithmetic, block design and comprehension. In earlier studies, these subtests were found to give a valid and reliable IQ estimate (reliability coefficient > .90) (Kaufman et al., 1996).

PDS: The Pubertal Development Scale (Petersen et al., 1988) measures the actual level of physical development during puberty. It is a 5-item self-report that measures items like body growth, body hair, skin changes for both sexes. For boys, there are items on beard growth and voice changes. For girls, there are items on breast growth and menstrual bleeding. Items can be answered on a 5-point scale with a total score range of 0-20. Internal consistency is adequate for both sexes, consistent across samples, while the predictive validity of the PDS is satisfactory (Robertson et al., 1992) The PDS was filled out by 92.3% of participants in this study.

Statistical analysis

Structural analysis. Besides the dimensional scales scores for unresolved loss or trauma and coherence of mind we used the categorical variable Ud-nondUd for unresolved-disorganized attachment versus resolved organized attachment in both the exploratory whole brain analysis, and the ROI analyses. No significant results were found.

Resting state analysis. The following pre-statistics processing was applied: motion correction (Jenskinson et al., 2002), non-brain removal (Smith et al., 2001), spatial smoothing using a Gaussian kernel of full-width-at-half-maximum 6.0 mm, and high-pass temporal filtering (highpass filter cutoff = 100.0 s). Functional scans were registered to the T1-weighted images, which were registered to standard space in order to calculate the transformation matrix for the higher-level group analysis (Jenkinson et al., 2002). The global signal was added to the model. It should be noted that there is no consensus regarding the global signal for resting state functional connectivity analyses (Murphy, & Fox, 2017). Adding the global signal to resting state analyses has both advantages and disadvantages. We added the global signal in the analyses of the current study in order to increase comparability with a previous resting state study with partly the same sample (Pannekoek et al., 2014b).

Functional connectivity Pre-statistics processing was applied before functional connectivity analyses, see supplemental material. A seed based correlation approach was used for the current study (Murphy, & Fox, 2007). We created a binary mask of the brain region that was significantly related to Unresolved loss and trauma: the left hippocampus. This region was used as seed region. After transforming the mask to native space, the mean time series for each participant were extracted from the left hippocampus. These times series were then used as a regressor in the model. In addition, CSF, white matter and the global signal (see supplemental material) were added as regressors to the model in order to reduce the influence of artifacts caused by physiological signal sources on the results (Fox, & Raichle, 2007). The temporal derivative of each regressor was added to the model resulting in 8 regressors in each model. Motion parameters were also added to the model.

Voxel-based morphometry. An exploratory whole brain analysis of the association of Unresolved loss or trauma with regional volume was performed with FSL-VBM (http://fsl.fmrib.o.ac.uk/fsl/fslwiki/FSLVBM) (Smith et al., 2004; Douaud et al., 2007). First, structural images were brain-extracted and gray matter-segmented before being registered to the MNI 152 standard space using non-linear registration. The resulting images were averaged and flipped along the x-axis to create a left-right symmetric, study-specific gray matter template. Second, all native gray matter images were non-linearly registered to this study-specific template and "modulated" to correct for local expansion (or contraction) due to the non-linear component of the spatial transformation. The modulated gray matter images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. After preprocessing steps, the association between unresolved loss or trauma and GMV was investigated using a GLM including the general psychopathology factor, sex, composite score age and pubertal status, total IQ score as confound regressors. A voxel-wise GLM was applied using permutation-based (5000 permutations) non-parametric testing and Threshold-Free Cluster Enhancement (Smith, & Nichols, 2009) was used to correct for multiple comparisons at the cluster level ($p<0.05$).

RESULTS

Voxel-based morphometry

VBM analyses did not show a significant relation between Unresolved status and gray matter volume.

Coherence of mind

We hypothesized that coherence of mind besides unresolved loss or trauma would correlate with a smaller hippocampal volume and a larger amygdala (Brown, & Morey, 2012; Brenning, & Braet, 2013), and that brain structures associated with coherence of mind would also show atypical functional connectivity. Similar to the analyses on Unresolved-disorganized attachment we analyzed whether coherence was associated with hippocampal and amygdalar volumes, and connectivities. No association was found between Coherence and hippocampal or amygdala volumes, nor with grey matter of the adolescent brain. Therefore, an association between Coherence and resting state functional connectivity (RSFC) was not further explored.

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Table S1. Mean (SD) general psychopathology scores for the internalizing, CSA-PTSD, and control group, and U versus nonU groups.

Group	N	M	SD	Range
Internalizing	21	0.46	0.83	-.88 – 2.34
CSA-PTSD	28	0.49	0.86	-.86 – 1.90
Control	25	-0.92	0.57	-1.66 – 0.39
U	16	0.38	.97	-1.31 – 1.90
nonU	58	-0.11	.99	-1.66 – 2.35

Table S2. Results of hierarchical regression analyses with hippocampal volume (L/R) and amygdala volume (L/R) as dependent variable, sex, age/pubertal status, total IQ score, and whole brain volume in the first step and Unresolved loss or trauma status (Ud versus non Ud/U continuous) in the second step.

	Ud-nonUd				U continuous			
	B	SE	ß	*p*	B	SE	ß	*p*
Left hippocampus	-269.99	110.15	-.27	.02	-237.39	154.57	-.18	.13
Right hippocampus	-195.87	119.96	-.19	.11	-267.52	164.14	-.19	.11
Left amygdala	-75.60	60.92	-.15	.22	-1.76	84.29	-.00	.98
Right amygdala	-103.40	73.91	-.17	.17	-29.17	102.50	-.04	.77

Table S3. Factor loadings for the Trauma Symptom Checklist for Children (TSCC), Children's Depression Inventory (CDI), Revised Child Anxiety and Depression Scale (RCADS), Adolescent Dissociative Experiences Scale (A-DES),Youth Self Report (YSR), and Child Behavior Check List (CBCL), resulting from the Principal Component Analysis.

Subscale	Loading
RCADS separation anxiety	.79
RCADS social phobia	.80
RCADS panic disorder	.69
RCADS generalized anxiety disorder	.74
RCADS obsessive compulsive disorder	.79
RCADS depressive disorder	.89
CDI	.84
YSR internalizing problems	.88
CBCL internalizing problems	.63
TSCC_depression	.92
TSCC anxiety	.75
TSCC_PTSD	.87
TSCC dissociation	.83
TSCC sexual concerns	.56
ADES	.70