



Amygdala habituation to emotional faces in adolescents with internalizing disorders, adolescents with childhood sexual abuse related PTSD and healthy adolescents



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ABSTRACT

Adolescents with internalizing disorders and adolescents with childhood sexual abuse related post-traumatic stress disorder (CSA-related PTSD) show a large overlap in symptomatology. In addition, brain research indicated hyper-responsiveness and sustained activation instead of habituation of amygdala activation to emotional faces in both groups. Little is known, however, about whether the same patterns of amygdala habituation are present in these two groups. The current study examined habituation patterns of amygdala activity to emotional faces (fearful, happy and neutral) in adolescents with a DSM-IV depressive and/or anxiety disorder (N = 25), adolescents with CSA-related PTSD (N = 19) and healthy controls (N = 26). Behaviourally, the adolescents from the internalizing and CSA-related PTSD group reported more anxiety to fearful and neutral faces than adolescents from the control group and adolescents from the CSA-related PTSD group reacted slower compared to the internalizing group. At the whole brain level, there was a significant interaction between time and group within the left amygdala. Follow-up ROI analysis showed elevated initial activity in the amygdala and rapid habituation in the CSA-related PTSD group compared to the internalizing group. These findings suggest that habituation patterns of amygdala activation provide additional information on problems with emotional face processing. Furthermore, the results suggest there are differences in the underlying neurobiological mechanisms related to emotional face processing for adolescents with internalizing disorders and adolescents with CSA-related PTSD. Possibly CSA-related PTSD is characterized by a stronger primary emotional response driven by the amygdala.

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1. Introduction

One of the most salient characteristics for social information processing is reading emotions from faces: multiple types of information, such as gender, age, emotional state and

trustworthiness are processed within several hundreds of milliseconds and provide crucial information for social interactions (Adolphs, 2002; Fusar-Poli et al., 2009; Grossmann and Johnson, 2007). Prior research has shown that the fusiform cortex and the amygdala are important brain regions involved in this process (Fusar-Poli et al., 2009), where the amygdala is often interpreted as a region involved in detecting the valence and intensity of expressed emotions (Costafreda et al., 2008; Whalen et al., 2009). Developmental neuroimaging studies reported that activity in this network is restructured in mid-adolescence (Casey et al., 2011), such that intensified emotion-processing makes the amygdala

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sensitive to reading emotions from faces of unknown others (Scherf et al., 2013). This is in line with several studies reporting that the amygdala shows stronger activity to emotional face processing in mid-adolescence compared to child- and adulthood (Guyer et al., 2008; Hare et al., 2008; Pfeifer et al., 2011; Somerville et al., 2011).

Research indicated that there are pronounced differences in amygdala activation between adults and adolescents with and without depression and anxiety disorders (also known as internalizing disorders). Several reports have shown that amygdala responsiveness is higher in healthy individuals who report high levels of depression or anxiety symptoms without being diagnosed with an internalizing disorder and in individuals diagnosed with an internalizing disorder (Monk et al., 2008a,b; Roberson-Nay et al., 2006; Somerville et al., 2004; Thomas et al., 2001; van den Bulk et al., 2014). These findings suggest that individuals with internalizing disorders demonstrate hypervigilant affective processing, which can be measured via individual differences in amygdala response to emotional faces.

Individuals who have a history of childhood maltreatment, such as childhood sexual abuse (CSA), also show increased patterns of amygdala activation (Garrett et al., 2012; Hart and Rubia, 2012). However, studies examining the neurobiological mechanisms of childhood maltreatment are scarce and many of the studies including adolescents who experienced childhood maltreatment, do not focus on CSA. There are only a few studies that investigated amygdala activation in adolescents with CSA-related PTSD. Research by Cisler et al. (2015) for example showed that adolescent girls who experienced physical or sexual assault and who were referred for trauma-focused cognitive behavioural therapy reacted differently to treatment based on their pre-treatment level of amygdala activation in response to threatening emotional faces: adolescents with a stronger amygdala response to emotional faces pre-treatment, showed less symptom reduction.

In addition, individuals who experienced CSA and who develop CSA-related Post-Traumatic Stress Disorder (PTSD) are at increased risk to develop subsequent internalizing disorders (Lindert et al., 2014), which makes internalizing disorders and CSA-related PTSD overlapping in symptomatology. This, in combination with the comparable levels of increased amygdala activation in response to emotional faces (Hart and Rubia, 2012; Monk et al., 2008a,b; Roberson-Nay et al., 2006; Thomas et al., 2001), highlights the need to investigate whether similar underlying neurobiological mechanisms are present in these two groups. To our knowledge, there is no research published that directly compared amygdala responsiveness in adolescents with an internalizing disorder and adolescents with CSA-related PTSD. It is possible that there are neurobiological differences between these two groups: although adolescents with internalizing disorders and adolescents with CSA-related PTSD show a large overlap in symptomatology (Lindert et al., 2014) and amygdala responsiveness to emotional faces (Hart and Rubia, 2012; Monk et al., 2008a,b), adolescents with CSA-related PTSD also have distinct characteristics like the experience of sexual abuse and a different attachment profile (van Hoof et al., 2015).

While it is challenging to reveal differentiating neurobiological mechanisms between adolescents with internalizing disorders and adolescents with CSA-related PTSD, one way to examine underlying distinct response patterns is by studying habituation effects. In healthy populations, the amygdala is known to habituate over time in response to emotional faces (Breiter et al., 1996; Fischer et al., 2003). The results of studies investigating habituation of amygdala activation in individuals with inhibited states, depression or anxiety are however inconsistent. For example, a study by Hare et al. (2008) showed that adolescents with higher levels of self-reported anxiety symptoms habituated more slowly to observing emotional faces than adolescents with lower levels of self-reported anxiety symptoms. However, this study did not include information

about the type of heightened self-reported anxiety and whether the adolescents experienced CSA. Two other studies reported relatively strong habituation effects in response to face processing within the amygdala, one in a sample of adults with a social anxiety disorder (Sladky et al., 2012) and another in a sample of female students scoring high on fear questionnaires (Wendt et al., 2012). Again, no information on CSA was available. To extend the current literature on habituation, CSA-related PTSD and internalizing disorders, it is of interest to compare amygdala habituation patterns in adolescents with internalizing disorders and adolescents with CSA-related PTSD.

In this study, we examined amygdala habituation in two groups known to show elevated amygdala responsiveness to emotional faces. We included individuals with a DSM-IV diagnosis of a depressive or anxiety disorder (INT group), adolescents with CSA-related PTSD (CSA-related PTSD group), and a matched healthy control group (CNTR group). Participants performed an emotional face-processing task validated in prior work (Monk et al., 2003; van den Bulk et al., 2013; van den Bulk et al., 2014), and we analysed the data for habituation patterns for subgroups of individuals by separating the task in three runs.

We aimed to investigate (1) whether the INT and CSA-related PTSD group show a deviant habituation pattern of amygdala activation compared to the CNTR group and (2) whether there are specific differences between the INT and CSA-related PTSD group in habituation patterns. We expected that the CNTR group shows fast habituation of amygdala activation (Breiter et al., 1996), that the INT and CSA-related PTSD group show increased amygdala activation in response to emotional faces (Garrett et al., 2012; McClure et al., 2007; Roberson-Nay et al., 2006) and that the INT group shows sustained activation of the amygdala (Hare et al., 2008). We were particularly interested in whether adolescents with CSA-related PTSD show a similar pattern of amygdala activation as adolescents with an internalizing disorder, or whether their neural patterns were dissociable, suggesting that although there is a large overlap in symptomatology between INT and CSA-related PTSD, a different underlying neurobiological mechanism is present.

2. Methods

2.1. Participants

Functional MRI data were collected for 31 healthy controls (CNTR group), 30 treatment naïve adolescents with a clinical diagnosis of a current DSM-IV depressive or anxiety disorder without CSA-related PTSD (INT group), and 22 treatment naïve adolescents with CSA-related PTSD (CSA-related PTSD group; comorbidity with anxiety and/or depression due to CSA was allowed). Of the original sample, 12 adolescents were excluded for the current analyses due to various reasons: technical problems during scanning ($N = 4$), excessive head movement (>3 mm; $N = 5$), unforeseen clinical features in the control group ($N = 1$), or anomalous findings reported by the radiologist ($N = 2$). The final sample consists of 26 CNTR adolescents, 26 INT adolescents and 19 adolescents with CSA-related PTSD (Table 1). All adolescents took part in the larger EPISCA study (Emotional Pathways' Imaging Study in Clinical Adolescents). Part of the data of the adolescents from the CNTR group and the INT group were presented before with a focus on test-retest reliability in healthy control group adolescents (van den Bulk et al., 2013) and on differences in amygdala activation between CNTR and INT adolescents and the relation with self-reported depression and anxiety symptoms (van den Bulk et al., 2014). The adolescents from the INT and CSA-related PTSD group were scanned before the start of regular treatment (Cognitive Behavioural Therapy (CBT) or Eye Movement Desensitization and Reprocessing (EMDR)).

Table 1

Participant characteristics of adolescents with an internalizing disorder, adolescents with CSA-related PTSD and control group adolescents. ^a = According to ADIS 18 met full PTSD criteria and one failed the interference criterion only slightly. ^b Univariate ANOVA's for CDI, RCADS anxiety and TSCC were corrected for age and IQ; * = questionnaire data was missing for one participant of the INT group and three participants of the CSA-related PTSD group; *** = questionnaire data was missing for three participants of the INT group and two participants of the CSA-related PTSD group; **** = questionnaire data was missing for three participants of the INT group and three participants of the CSA-related PTSD group; CNTR = control group, CLIN = INT and CSA-related PTSD group, IQ = Intelligence Quotient, GAD = Generalized Anxiety Disorder, SAD = Social Anxiety Disorder, NOS = Not Otherwise Specified, CDI = Children's Depression Inventory, RCADS = Revised Children's Anxiety and Depression Scale.

	INT	CSA-related PTSD	CNTR				
	N	N	N	χ^2	df	<i>p</i>	
N	26	19	26				
Females/Males	22/4	17/2	23/3	0.282	2	0.868	
	Mean _(sd;range)	Mean _(sd;range)	Mean _(sd;range)	F	df	<i>p</i>	
Age	15.98 _(1.45;13.23–17.99)	16.62 _(1.79;13.82–20.57)	15.25 _(1.64;13.17–19.82)	4.02	2,70	0.022	CSA > CNTR
Full scale IQ	105.12 _(8.66;87–119)	99.89 _(9.10;87–114)	106.58 _(7.77;90–122)	3.63	2,70	0.032	CNTR > CSA
<i>Clinical assessment based diagnosis:</i>	N _(%)	N _(%)	N _(%)				
No disorders	0	0	26 _(100%)				
Depression	7 _(27%)	0	0				
Dysthymia	10 _(38%)	0	0				
GAD	3 _(11.5%)	0	0				
SAD	2 _(8%)	0	0				
Anxiety NOS	1 _(4%)	0	0				
Adjustment disorder with dep./anx.	3 _(11.5%)	0	0				
CSA-related PTSD	0	19 _(100%)	0				
<i>ADIS based PTSD classification:</i>							
No PTSD	19 _(73%)	1 _{(5%)^a}	26 _(100%)				
PTSD (sexual abuse)	0	16 _(84%)	0				
PTSD (other cause)	7 _(27%)	0	0				
PTSD (sexual abuse + other cause)	0	2 _(11%)	0				
<i>Self-reported symptomatology^b</i>	Mean _(sd;range)	Mean _(sd;range)	Mean _(sd;range)	F _(group)	df	<i>p</i>	
CDI: total score ^c	19.06 _(9.10;2–40)	15.92 _(7.12;5–28)	4.56 _(3.40;0–13)	30.62	2,66	<0.001	CLIN > CNTR
RCADS: total score anxiety subscales ^d	31.84 _(14.16;3–70)	34.69 _(14.36;12–57)	14.85 _(10.83;0–40)	15.84	2,65	<0.001	CLIN > CNTR
TSCC: total score ^e	42.51 _(22.67;7–98)	44.31 _(21.69;14–83)	17.63 _(13.80;0–44)	12.99	2,64	<0.001	CLIN > CNTR

^a According to ADIS 18 met full PTSD criteria and one failed the interference criterion only slightly.

^b Univariate ANOVA's for CDI, RCADS anxiety and TSCC were corrected for age and IQ; .

^c Questionnaire data was missing for one participant of the INT group and three participants of the CSA-related PTSD group.

^d Questionnaire data was missing for three participants of the INT group and two participants of the CSA-related PTSD group.

^e Questionnaire data was missing for three participants of the INT and three participants of the CSA-related PTSD group; CNTR = control group, CLIN = INT and CSA-related PTSD group, IQ = Intelligence Quotient, GAD = Generalized Anxiety Disorder, SAD = Social Anxiety Disorder, NOS = Not Otherwise Specified, CDI = Children's Depression Inventory, RCADS = Revised Children's Anxiety and Depression Scale.

Adolescents from the INT and CSA-related PTSD group were recruited in outpatient departments of three child and adolescent psychiatric institutes in the surrounding area of Leiden. Inclusion criteria for participants in the INT group were: having a clinical diagnosis of any DSM-IV depressive or anxiety disorder, no experience of CSA, being referred for regular CBT-like psychotherapy, and being treatment naïve. Inclusion criteria for the CSA-related PTSD group were: having lifetime experiences of sexual abuse by one or more perpetrators in- or outside the family and being referred for CBT-like or EMDR therapy. Adolescents in the CNTR group were recruited through local advertisements, with the following inclusion criteria: no clinical scores on validated mood and behavioural questionnaires, no history of traumatic experiences and no current psychotherapeutic intervention of any kind. All adolescents were between 12 and 21 years of age and had an estimated intelligence ≥ 80 . Exclusion criteria for all participants were: any other primary DSM-IV diagnosis, current use of psychotropic medication (except for stable SSRI use; $N=4$), current substance abuse, a history of neurological disorders or severe head injury, left-handedness, and general MRI contraindications.

For all participants, estimated full-scale IQ scores were acquired with six subtests of the Wechsler Intelligence Scale for Children-III or the Wechsler Adult Intelligence Scale (Wechsler, 1991, 1997). There was a significant difference between groups in age ($F_{(2,70)} = 4.02, p < 0.05$) and IQ ($F_{(2,70)} = 3.63, p < 0.05$) but not for sex distribution ($\chi^2_{(2)} = 0.282, p = 0.87$). Post hoc comparisons showed that the CSA-related PTSD group was significantly older and had a significant lower IQ score than the CNTR group ($p < 0.05$ and $p < 0.05$ respectively). The INT group did not differ in age and IQ from the

CNTR ($p = 0.32$ and $p = 1.00$) and CSA-related PTSD group ($p = 0.60$ and $p = .14$). Because of the significant difference in age and IQ, these variables were included as covariates in all analyses.

After complete description of the study to the participants, informed consent was obtained from all participants, and from a primary care giver for every participant under the age of 18. The adolescents received a financial compensation including travel expenses for participating in the study. The Medical Ethics Committee of the Leiden University Medical Centre approved the study and all anatomical scans were reviewed and cleared by a radiologist.

2.2. Clinical assessment

First, all adolescents from the INT and CSA-related PTSD group were clinically assessed by a child and adolescent psychiatrist as part of the standard intake/interview procedures. After they were diagnosed with a depressive or anxiety disorder or CSA-related PTSD, they were asked to participate in the study. As part of the study protocol, the child and parent versions of the Anxiety Disorders Interview Schedule (ADIS; Silverman and Albano, 1996) were used to obtain DSM-IV-based classifications of depressive and anxiety disorders and PTSD. Standardized dimensional measures were used for assessing the severity of self-reported symptoms of depression, anxiety and trauma; i.e. the total score of the Children's Depression Inventory (CDI; Kovacs, 1992), the total anxiety scale of the Revised Children's Anxiety and Depression Scale (RCADS; Chorpita et al., 2000) and the total score of the Trauma Symptom Checklist for Children (TSCC; Briere, 1996). The same measures were assessed in the CNTR group, and participants were excluded

if they met the criteria for a DSM-IV diagnosis based on the ADIS-interviews or scored in the clinical range questionnaires assessing symptomatology. We used expectation maximization when items in the CDI (8 items across all participants), the RCADS (4 items across all participants) and the TSCC (6 items across all participants) were missing.

2.3. Task

All participants performed an emotional face-processing task (McClure et al., 2007; Monk et al., 2003), which was described in detail previously (van den Bulk et al., 2013; van den Bulk et al., 2014). In short, the task consisted of three randomly presented constrained conditions ('how afraid are you?', 'how happy are you?' and 'how wide is the nose?') and one unconstrained condition (passive viewing). Within each run (three in total), all four conditions were presented in a random order. After the presentation of a condition, which included instructions for the following trials, participants viewed 21 pictures expressing a fearful, neutral or happy face (a total of 21 trials per condition, presented in a random order), which they had to rate on a four-point rating scale (1. not at all, 2. a little, 3. quite and 4. very). The ratings were dependent on the beforehand-presented condition. Reaction times and ratings of the different emotional faces (fearful, happy or neutral) were recorded. The task used a mixed design and different conditions were included to divert attention towards or away from features of the face that provide information for emotion processing.

All trials had the same structure: first participants were presented with one of the conditions for 4000 milliseconds, followed by a fixation cross with a jittered duration between 500 and 6000 milliseconds. Thereafter, a picture was shown for 3000 milliseconds during which participants had to rate their emotion (Fig. 1). The presentation of the fixation crosses and the pictures was repeated for 21 trials, after which the next condition was presented. Trials during which the participants did not respond within 3000 milliseconds (1.91% in total) were not included in the behavioural analyses and they were included as regressor of no interest in the fMRI analyses.

Since we were interested in habituation effects, we modelled the three runs separately. To be sure that enough trials were present per emotion, we collapsed across conditions and only focused on emotional valence (fearful, happy and neutral). This resulted in 28 trials for each emotion for each run.

2.4. Image acquisition

Data were acquired using a 3.0T Philips Achieva (Philips, Best, The Netherlands) scanner at the Leiden University Medical Centre. First, a localizer was obtained for each participant. Subsequently, T2*-weighted Echo-Planar Images (EPI) (TR=2200 ms, TE=30 ms, flip angle=80°, 80 × 80 matrix, FOV=220 mm, 38 slices of thickness 2.72 mm) were obtained during three functional runs of 192 vols each. Each run had two additional scans at the start, which were discarded to allow for equilibration of T1 saturation effects. Also, a sagittal 3-dimensional gradient-echo T1-weighted image was acquired with the following scan parameters: TR=9.8 ms; TE=4.6 ms; flip angle=8°; 192 × 152 matrix; FOV=224 × 177 × 168 mm, 140 sagittal slices; no slice gap; 1.16 × 1.16 × 1.20 mm voxels. Stimuli were presented onto a screen located at the head of the scanner bore and viewed by participants by means of a mirror mounted to the head coil assembly. Participants could indicate their response by using a button box that was attached to their legs.

2.5. Analyses

2.5.1. Behaviour

To examine differences between groups in self-reported depression, anxiety and trauma symptomatology, we performed three univariate ANOVAs including age and IQ as covariates. Post hoc comparisons were Bonferroni corrected. For the subjective scoring of emotional faces we performed three separate analyses with run (1–3) and emotion (fearful, happy, neutral) as within-subject factors and group as a between subjects factor using repeated measurement ANOVAs in SPSS 19. The scores were analysed separately for each state question, because values of the scores represent different interpretations for each question. Reaction times were analysed with one repeated measurement ANOVA including run (3 levels), constrained condition (3 levels) and emotion (3 levels) as within subject factors and group as between-subjects factor. We included age and IQ as covariates in the repeated measurement analyses and all post hoc comparisons were Bonferroni corrected. In case sphericity could not be assumed, a Greenhouse–Geisser correction (GG-corr.) was used.

2.5.2. Whole brain

The collected data were analysed using SPM8 (Wellcome Department of Cognitive Neurology, London). Functional time series were realigned to compensate for small head movements and differences in slice timing acquisition. Functional volumes were first registered and normalized onto the individual structural T1 and thereafter to the T1 template. The normalization algorithm used a 12-parameter affine transformation together with a nonlinear transformation involving cosine basis functions and resampled the volumes to three mm. cubic voxels. Functional volumes were spatially smoothed with an 8 mm, full-width at half-maximum isotropic Gaussian kernel. The MNI (Montreal Neurological Institute) 305 stereotaxic space templates (Cocosco et al., 1997) were used for visualization and all results are reported in this template, which is an approximation of Talairach space (Talairach and Tournoux, 1988).

Individual subjects' data were analysed using the general linear model in SPM8. The fMRI time series were modelled by a series of events convolved with a canonical hemodynamic response function (HRF). The state questions were modelled separately as 4000 millisecond events and included as covariates of no interest. The picture presentation of each emotional face was modelled as a zero duration event. In the model, the picture presentation was further divided in nine separate functional trials (three runs by three emotions). The modelled events were used as a covariate in a general linear model along with a basic set of cosine functions that high-pass filtered the data. The least squares parameter estimates of the height of the best-fitting canonical HRF for each condition were used in pair wise contrasts (i.e. all faces > fixation, run1 > run3). The resulting contrast images, computed on a subject-by-subject basis, were submitted to group analyses. At the group level, the contrasts were computed by performing a full-factorial model with group as a three-level factor and age and IQ as covariates, treating subjects as a random effect. Task- and habituation related responses were considered significant if they consisted of at least 10 contiguous voxels at a FWE-corrected threshold of $p < 0.05$.

2.5.3. ROI

Based on the current literature on face processing and habituation we used a priori ROI selection of bilateral amygdala to test our hypotheses on habituation. We used the MarsBaR toolbox for use with SPM8 (<http://marsbar.sourceforge.net/>); (Brett et al., 2002) to perform region interest (ROI) analyses. We defined the ROIs in two different ways. First, we used the functional regions identified in the unbiased contrast all faces vs. fixation of the whole-brain

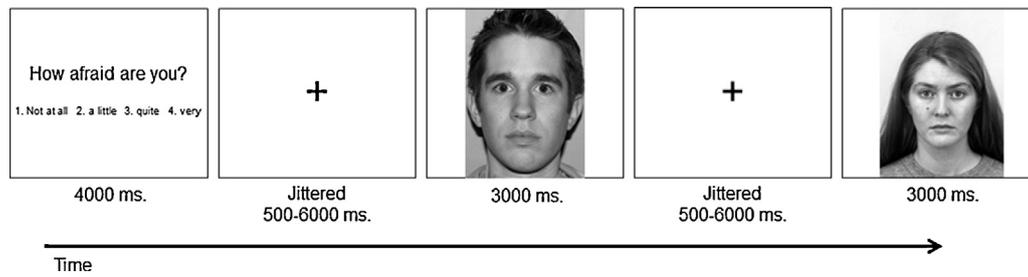


Fig. 1. Visual representation of the emotional face-processing task. Participants were first presented with one of the conditions (i.e., how happy are you, how afraid are you, how wide is the nose or passive viewing) followed by a fixation cross. Thereafter, twenty-one pictures with a negative, positive or neutral face was shown (random selection) during which participants had to rate the pictures (1 = not at all, 4 = very). The pictures were alternated with a fixation cross.

analyses ($N=71$; FWE corrected, $p < 0.05$, at least 10 contiguous voxels). This resulted in the right amygdala ROI. The left amygdala ROI was derived from the same contrast but with whole brain FDR instead of FWE correction, because it was not significantly active at the stringent whole brain FWE threshold. The left amygdala ROI spanned several functional brain regions and therefore was subdivided by sequentially masking the functional ROI with the anatomical MarsBaR ROI. The advantage of this approach is that the ROIs only include voxels within the amygdala that are engaged during the task. Second, we used the anatomical template for left and right amygdala ROI as provided by MarsBaR. This way, ROI selection is not influenced by the potentially additional contribution from one of the groups included in the study. The percent signal change values of the four ROIs were further analysed using two 2 (lateralization) \times 3 (runs) \times 3 (emotions) repeated measurement ANOVA in SPSS 19 and all post hoc comparisons were Bonferroni corrected. We only included age and IQ as covariates in the ROI analyses performed in SPSS, the whole brain analyses from which we derived the ROIs were not corrected for age and IQ.

2.5.4. Multiple regression

To further examine the relation between self-reported symptomatology, the presence CSA-related PTSD and amygdala activation/habituation, we performed four multiple regression analyses (ENTER method, hierarchical), in which we included amygdala activation in run 1 or amygdala habituation between run1-run3 as dependent variables and age and IQ as covariates in step 1 and the raw score for self-reported depression, anxiety and trauma symptoms, the presence of CSA-related PTSD as independent variables in step 2. Separate analyses were performed for left and right amygdala activation. We inspected the correlation matrix and the tolerance values to examine multicollinearity between the independent variables. There were no correlations above 0.90 and tolerance values were all above 0.10, suggesting there was no problematic multicollinearity between the independent variables.

3. Results

3.1. Behavioural data

3.1.1. Self-reported levels of depression, anxiety and trauma symptoms

The univariate ANOVA for self-reported levels of depression (CDI) resulted in a significant effect for group ($F_{(2,66)} = 30.62$, $p < 0.001$, $\eta^2 = 0.50$) in which the INT group and the CSA-related PTSD group scored significantly higher than the CNTR group (both p 's < 0.001). There was no significant difference between the INT and CSA-related PTSD group ($p = 0.99$). For the RCADS anxiety scale and the TSCC total scale comparable results were obtained: a significant effect of group ($F_{(2,65)} = 15.84$, $p < 0.001$, $\eta^2 = 0.34$ and $F_{(2,64)} = 12.99$, $p < 0.001$, $\eta^2 = 0.30$ respectively) in which the INT

group and the CSA-related PTSD group scored significantly higher than the CNTR group (all p 's < 0.001). Again there was no significant difference between the INT and CSA-related PTSD group for self-reported anxiety ($p = 1.00$) and trauma ($p = 1.00$) symptoms. See Table 1 for descriptives and test statistics.

3.1.2. Subjective rating of emotional faces

The repeated measurement ANOVA for the condition 'how afraid are you?' resulted in a main effect of group ($F_{(2,64)} = 4.19$, $p < 0.05$, $\eta^2 = 0.12$) and an emotion \times group interaction effect ($F_{(4,128)} = 3.29$, $p < 0.05$, $\eta^2 = 0.09$). This interaction revealed that the INT adolescents ($p < 0.05$) and the adolescents with CSA-related PTSD ($p < 0.05$) endorsed higher self-reported fear to fearful faces than the CNTR adolescents. For happy and neutral faces there were no significant differences between groups (all p 's > 0.10 ; see Fig. 2).

The ANOVA for the condition 'how happy are you?' resulted in a main effect of group ($F_{(2,62)} = 5.56$, $p < 0.01$, $\eta^2 = 0.15$), but no group \times emotion interaction. The main effect of group showed that the overall subjective happiness ratings of the CNTR group was higher than for the INT group ($p < 0.01$), whereas the CSA-related PTSD group did not differ from the INT or CNTR group (both p 's > 0.15).

Finally, the ANOVA for the condition 'how wide is the nose?' resulted in a main effect of emotion ($F_{(2,130)} = 5.98$, $p < 0.005$, $\eta^2 = 0.08$), with higher subjective scoring for happy and fearful faces compared to neutral faces (both p 's < 0.001), and higher subjective scoring for happy than for fearful faces ($p < 0.001$). There was no main/interaction effect with group.

There was no main or interaction effect of run in any of the conditions suggesting an absence of habituation at the behavioural level.

3.1.3. Reaction times

The ANOVA for reaction time resulted in a main effect of group ($F_{(2,62)} = 5.13$, $p < 0.01$, $\eta^2 = 0.14$) and an emotion \times run \times group interaction ($F_{(8,248)} = 2.67$, $p < 0.01$, $\eta^2 = 0.08$). Post hoc comparisons showed higher reaction times for the CSA-related PTSD group compared to the INT group ($p < 0.01$). There were no significant differences between the CSA-related PTSD and CNTR groups ($p = 0.20$) and the CNTR and INT groups ($p = 0.47$). Post hoc comparisons for the three-way interaction showed that the CSA-related PTSD group had higher reaction times than the INT group for fearful faces in run 1 ($p < 0.005$) and run 2 ($p < 0.05$), for happy faces in run 2 ($p < 0.05$) and run 3 ($p < 0.05$) and for neutral faces in run 1 ($p < 0.005$) and run 3 ($p < 0.005$).

3.2. Whole brain analyses

The whole brain analysis for all faces $>$ fixation resulted in robust activation in right amygdala across participants (Fig. 3A). The contrast run1 $>$ run3 resulted in significant activation in bilateral amygdala, suggesting changes in amygdala activation over time

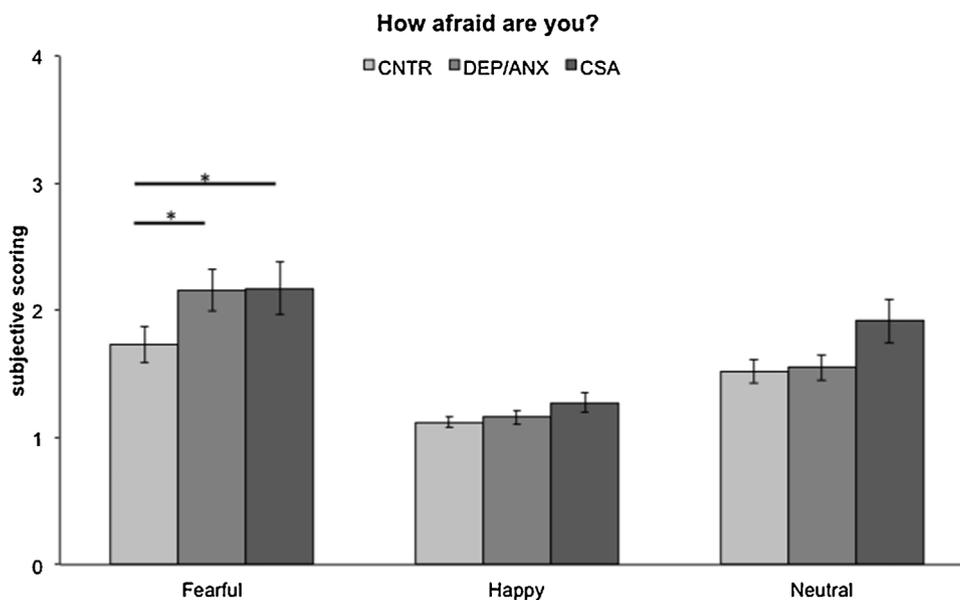


Fig. 2. Group differences in subjective scoring of emotional faces with the ‘How afraid are you?’ condition. Results were collapsed across runs. The INT and CSA-related PTSD group reported being more afraid for fearful faces than the CNTR group. * $p < 0.05$; CNTR = control group; INT = internalizing adolescents; CSA = adolescents with CSA-related PTSD.

across participants. To follow-up the run effect, we inspected the main effect of group within the contrast run1 > run3 (i.e., a group \times time interaction). The results showed a significant group effect specifically in the left amygdala (uncorrected, $p < 0.001$, 10 voxels, no regions were detected when applying FDR or FWE correction; Fig. 3B). Follow up t -tests for the contrast run1 > run3 for each group separately revealed activation in this region only for the CSA-related PTSD group ($p < 0.001$ uncorrected). These findings suggest differences between groups in habituation patterns in the left amygdala when testing across the whole brain. The patterns across runs for the three groups were examined in detail using region of interest since region of interest analyses typically have more power to detect small group differences.

3.3. Region of interest analyses

3.3.1. Functionally defined ROIs

The repeated measurement ANOVA for left and right amygdala activation resulted in a run \times group interaction ($F_{(4,132)} = 3.66$, $p < 0.01$, $\eta^2 = 0.10$). Post hoc comparisons showed an initial heightened response of amygdala activation in run 1 in the CSA-related PTSD group compared to the INT group ($p < 0.005$) and a borderline significance compared to the CNTR group ($p = 0.058$). Furthermore, for the CSA-related PTSD group there was a significant decrease in activation between run1 and run2 ($p < 0.005$), between run1 and run3 ($p < 0.001$) and between run2 and run3 ($p < 0.05$). A comparable pattern of a decrease in amygdala activation was seen for the CNTR group: run1–run2, $p < .05$ and run1–run3, $p < .05$. For the INT group there were no significant in- or decreases in amygdala activation over runs (p 's > .10). See also Fig. 4.

3.3.2. Anatomically defined ROIs

The repeated measurement ANOVA resulted in a significant run \times group interaction ($F_{(4,132)} = 3.03$, $p < 0.05$, $\eta^2 = 0.08$). Post hoc comparisons showed an initial heightened response of amygdala activation in run 1 in the CSA-related PTSD group compared to the INT group ($p < 0.05$). Furthermore, for the CSA-related PTSD group there was a significant decrease in activation between run1 and run2 ($p < .005$), between run1 and run3 ($p < .001$). For the CNTR and

INT group there were no significant in- or decreases in amygdala activation over runs (p 's > .10).

Overall, there was no main/interaction effect for lateralization or emotion, suggesting that the results are present for both left and right amygdala and for all three emotions.

3.4. Multiple regression analyses

We included amygdala activation in run 1 or amygdala habituation between run1–run3 as dependent variables and age and IQ as covariates in step 1 and the raw score for self-reported depression, anxiety and trauma symptoms and the presence of CSA-related PTSD as independent variables in step 2. Separate analyses were performed for left and right amygdala activation. The multiple regression analyses showed that the self-reported depression, anxiety, trauma symptomatology questionnaires and the presence of CSA-related PTSD predicted left amygdala activation (model 2, $F_{(6,64)} = 2.27$, $p < 0.05$, $R^2 = 0.19$) and left amygdala habituation (model 2, $F_{(6,64)} = 2.49$, $p < 0.05$, $R^2 = 0.21$). Only the presence of CSA-related PTSD significantly predicted left amygdala activation ($\beta = 0.48$, $t_{(58)} = 3.56$, $p < 0.005$) and left amygdala habituation ($\beta = 0.40$, $t_{(58)} = 2.97$, $p < 0.005$). The depression, anxiety and trauma questionnaires did not significantly predict left amygdala activation (p 's > 0.75) or habituation (p 's > 0.19). For right amygdala, the regression model was not significant: activation (model 2, $F_{(6,64)} = 1.57$, $p = 0.17$, $R^2 = 0.14$), habituation (model 2, $F_{(6,64)} = 1.40$, $p = 0.23$, $R^2 = 0.13$). However, when we evaluated the independent variables, presence of CSA-related PTSD was the only significant predictor: amygdala activation $\beta = 0.32$, $t_{(58)} = 2.31$, $p < 0.05$, amygdala habituation $\beta = 0.33$, $t_{(58)} = 2.33$, $p < 0.05$. See Table 2 for all regression coefficients. The other variable, depression, anxiety and trauma symptoms, did not significantly predict right amygdala activation (p 's > 0.38) or habituation (p 's > 0.39).

4. Discussion

The goal of this study was to examine whether amygdala habituation during an emotional face-processing task differed between adolescents with an internalizing disorder and adolescents with CSA-related PTSD, and to compare the patterns of amygdala

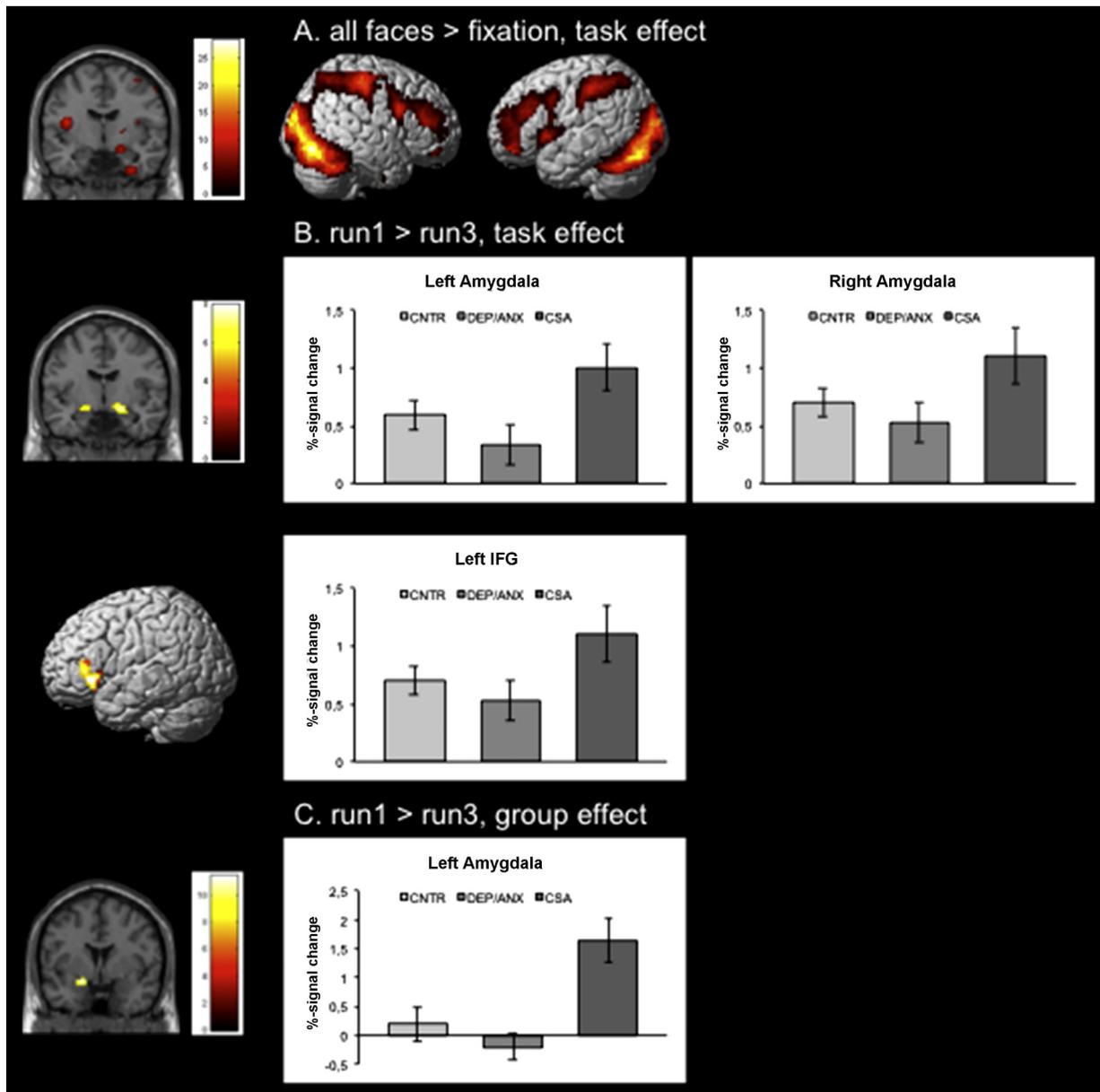


Fig. 3. Overview of whole brain results derived from a full factorial model including three groups and three runs. A. the contrast all emotional faces > fixation for the main effect of the task (FWE corrected, $p < 0.05$; 10 contiguous voxels), B. the contrast emotional faces in run 1 > emotional faces in run3 for the main effect of the task (FWE corrected, $p < 0.05$; 10 contiguous voxels) and C. the contrast emotional faces in run 1 > emotional faces in run3 for the main effect of group (uncorrected, $p < 0.001$; 10 contiguous voxels). Left and right amygdala (represented in B) were followed-up by one ROI analysis to visualize the direction of the effects. CNTR = control group; INT = internalizing adolescents; CSA = adolescents with CSA-related PTSD.

habituation with healthy control group adolescents. This is important because adolescents with internalizing disorders show only partial overlap in symptomatology with adolescents with CSA-related PTSD (Lindert et al., 2014), with the latter experienced childhood sexual abuse and subsequently developed PTSD. Possibly, the experience of CSA relates to different neurobiological mechanisms for emotion processing.

Consistent with prior studies (Breiter et al., 1996; Fischer et al., 2003), healthy adolescents showed a habituation effect in the amygdala when viewing emotional faces: activation in the amygdala was significantly higher during run1 then during run2/run3. This effect was present for all emotional faces, so not solely for fearful faces, which is in line previous studies (Breiter et al., 1996). This might suggest that habituation to emotional faces may be a general pattern that is related to, for example, the novelty of the emotional faces which adapts over time. Previous research showed that

amygdala response for novel neutral faces is larger than for familiar neutral faces, but in both cases amygdala activation declined over time (Schwartz et al., 2003). Therefore, Schwartz et al. (2003) suggest that one function of the amygdala is to detect new events that might be important.

Within the INT and CSA-related PTSD group, habituation-related amygdala activity showed different patterns. For the CSA-related PTSD group we found initial increased activation in the amygdala and relatively fast habituation to a level comparable to that of the INT and CNTR group. In the INT group we did not find significant habituation effects in the amygdala. Instead, these adolescents showed comparable levels of amygdala activation as the CNTR group but there was no significant habituation of activation. In addition, the results showed a difference between the INT and CSA-related PTSD group in amygdala activation: the CSA-related PTSD group had a higher amygdala response in run 1 compared to

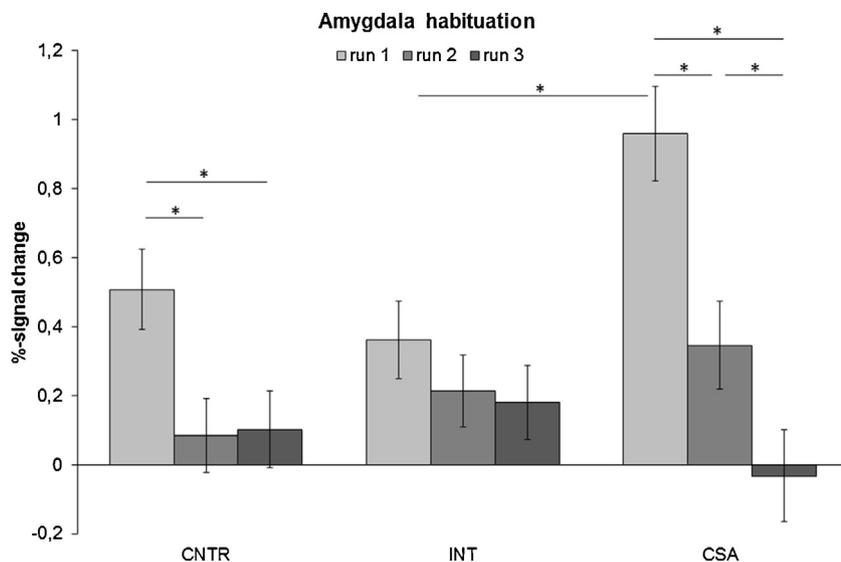


Fig. 4. Region of interest analysis for left and right amygdala activation over runs, collapsed across emotions. Regions were derived from the contrast all emotional faces > fixation with a FWE correction for right amygdala ($p < 0.05$; 10 contiguous voxels) and a FDR correction for left amygdala ($p < 0.05$; 10 contiguous voxels). * $p < 0.05$; CNTR = control group; INT = internalizing adolescents; CSA = adolescents with CSA-related PTSD.

the INT group. These results were still significant when we excluded the seven participants from the INT group that fulfilled the PTSD criteria on the ADIS interview, suggesting that the reported effect is specific for CSA-related PTSD. In addition to the functionally defined amygdala ROIs, we also included anatomically defined ROIs of the amygdala to make sure the results were not influenced by the potentially additional contribution from one of the groups included in the study. Again there was a significant interaction between run and group in which the CSA-related PTSD group showed an initial heightened amygdala response and relatively fast habituation. Contrary to the results of the functionally defined ROIs, we did not find habituation effects in the CNTR and INT group. Possibly, the habituation effects for the CNTR group are only present in the more specific and task related functionally defined ROIs.

To confirm the interpretation, the results of the ROI analysis were followed-up by regression analyses investigating whether self-reported anxiety, depression and trauma symptoms or the presence of CSA-related PTSD predicted amygdala activation and amygdala habituation within the complete sample. The results of these analyses showed that only presence of CSA-related PTSD predicted (especially left) amygdala activation and habituation. This increases the robustness of the whole brain and ROI analyses and suggests that the adolescents with CSA-related PTSD show a distinct pattern of amygdala habituation in response to emotional faces.

Contrary to prior reports (McClure et al., 2007; Monk et al., 2008a,b; Thomas et al., 2001), the INT group did not show a general higher amygdala response to emotional faces than the CNTR adolescents. This finding was surprising, however, we previously reported that self-reported levels of anxiety and not diagnosis per se predicted amygdala activation (van den Bulk et al., 2014). Possibly, individual differences in depression and anxiety symptomatology or the inclusion of different types of anxiety disorders suppressed group differences in amygdala activation. Another explanation can be found in the current task design: we used a task design in which participants rated their subjective feeling while some studies have shown that attention load (such as answering questions or rating the emotional faces) influences amygdala activation (Costafreda et al., 2008; Sauer et al., 2013). We did include a passive viewing condition. However, not enough trials were available to examine habituation during passive viewing. Future research should further

investigate habituation, for example, by using a passive viewing task with a sufficient number of trials per run. Furthermore, it would be interesting to further examine the effect of constrained and unconstrained conditions on amygdala activation. In the current study, we collapsed the analyses across state questions to have enough trials left for the habituation analyses, which prevented us from examining the effect of state questions on amygdala activation and habituation. Future research should further examine the effect of state questions. To do so, it might be interesting to include two face processing tasks: one in which participants have to passively view emotional faces and another one in which participants have to regulate their emotions or have to answer questions in response to the emotional faces. The results of these two tasks can then be compared and more knowledge can be acquired about the influence of cognitive load on amygdala activation and habituation. Finally, future research should include larger samples with a wide variety of subtypes of anxiety disorders and an equal distribution of males/females.

The innovative aspect of the current study was that we included both adolescents with internalizing disorders and adolescents with CSA-related PTSD. Although the overlap in reported symptomatology between these groups is high, the adolescents with CSA-related PTSD experienced childhood sexual abuse and subsequently developed CSA-related PTSD. Previous research has demonstrated that people who experienced childhood maltreatment show heightened patterns of amygdala activation (Hart and Rubia, 2012; van Harmelen et al., 2013) and that experiencing childhood maltreatment often leads to the development of CSA-related PTSD in combination with internalizing disorders (Lindert et al., 2014). With respect to the behavioural data (subjective scoring of emotional faces), we showed that adolescents who experienced CSA report the same elevated level of fear to fearful faces as depressed/anxious adolescents. However, they responded slower to the emotional faces compared to the INT or CNTR group on neurobiological level adolescents with CSA-related PTSD showed higher amygdala activation at the beginning of the task (initial increase) compared to CNTR group and INT group, but similar activation as CNTR group near the end of the task (relatively fast habituation).

There are some potential interpretations for the different habituation effects between internalizing adolescents and adolescents

Table 2

Regression coefficients from multiple regression analyses. Left amygdala ROI was derived from the contrast all emotional faces > fixation with a whole brain FDR correction ($p < 0.05$; 10 contiguous voxels), right amygdala ROI was derived from the contrast all emotional faces > fixation with a whole brain FWE correction ($p < 0.05$; 10 contiguous voxels). IQ = Intelligence Quotient, CDI = Children's Depression Inventory, RCADS = Revised Children's Anxiety and Depression Scale.

	F(df)	p-value	R ²	B	SE B	β
Model 1: left amygdala activation	0.061(2,64)	0.941	0.002			
Constant				0.215	1.41	
Age				-0.004	0.051	-0.010
IQ				0.003	0.010	0.042
Model 2: left amygdala activation	2.27(6,64)	0.049	0.19			
Constant				-0.170	1.369	
Age				-0.051	0.050	-0.128
IQ				-0.013	0.010	0.163
CDI				0.005	0.015	0.062
RCADS				-0.003	0.012	-0.074
TSCC				-0.001	0.009	-0.022
CSA-related PTSD present				0.763	0.214	.482*
Model 1: left amygdala habituation	1.56(2,64)	0.218	0.048			
Constant				-0.962	1.958	
Age				0.118	0.071	0.209
IQ				-0.005	0.014	-0.043
Model 2: left amygdala habituation	2.49(6,64)	0.032	0.21			
Constant				-1.291	1.936	
Age				0.065	0.070	0.115
IQ				0.005	0.014	0.044
CDI				0.020	0.021	0.188
RCADS				0.008	0.016	0.133
TSCC				-0.016	0.012	-0.373
CSA-related PTSD present				0.899	0.303	.399*
Model 1: right amygdala activation	0.697(2,64)	0.502	0.022			
Constant				2.223	1.304	
Age				-0.037	0.047	-0.099
IQ				-0.009	0.009	-0.126
Model 2: right amygdala activation	1.57(6,64)	0.172	0.14			
Constant				1.781	1.323	
Age				-0.064	0.048	-0.172
IQ				-0.003	0.010	-0.036
CDI				0.006	0.015	0.088
RCADS				0.009	0.011	0.224
TSCC				-0.007	0.008	-0.262
CSA-related PTSD present				0.478	0.207	.323*
Model 1: right amygdala habituation	0.513(2,64)	0.601	0.016			
Constant				1.140	1.707	
Age				0.029	0.062	0.061
IQ				-0.010	0.012	-0.104
Model 2: right amygdala habituation	1.40(6,64)	0.229	0.13			
Constant				0.589	1.740	
Age				-0.004	0.063	-0.009
IQ				-0.001	0.013	-0.013
CDI				0.006	0.019	0.062
RCADS				0.011	0.015	0.200
TSCC				-0.009	0.011	-0.257
CSA-related PTSD present				0.635	0.272	.328*

1Results were highly comparable when the 7 adolescents from the INT group that fulfilled the criteria for PTSD on the ADIS interview were excluded: interaction run x group ($F_{(4,118)} = 3.81, p < 0.01, \eta^2 = 0.11$), CSA-related PTSD > INT in run 1 ($p < 0.01$), CSA-related PTSD run1 > run2 ($p < 0.005$), CSA-related PTSD run2 > run3 ($p < 0.001$), CSA-related PTSD run1 > run3 ($p < 0.05$), CNTR run 1 > run2 ($p < 0.01$), CNTR run1 > run3 ($p < 0.05$). There were no significant effects within the INT group.

with CSA related PTSD. For example, higher arousal in adolescents with CSA-related PTSD may result in increased vigilance to information from outside, while the integration of information in the cognitive control regions (e.g. top-down regulation by the medial PFC) is intact, resulting in a heightened amygdala response that does habituate over time. In internalizing adolescents this might be different: the primary emotional response is less exaggerated but maybe the integration of information by cognitive control regions is insufficient. Future studies should replicate the current effects to demonstrate if these habituation patterns are also found in other studies focusing on CSA-related PTSD, for example by reanalysing existing data sets. Furthermore, larger samples should be included to be able to investigate habituation effects in subtypes of anxiety and depressive disorders. In addition, tasks should be used to examine neurobiological mechanisms involved in emotion regulation in internalizing adolescents and adolescents with CSA-related PTSD.

Even though we aimed to include a comprehensive sample with a well-validated experimental task, several limitations of this study need to be mentioned. First, we had to collapse across conditions within the emotional face-processing task to have enough power left for the habituation analyses. This limits the degree to isolate specific task effects and possibly suppressed current findings. In addition, not including the conditions in the fMRI analyses prevents us to directly compare the behavioural results with the neural results. At a behavioural level, we found that INT adolescents and adolescents with CSA-related PTSD reported being more afraid for fearful faces and less happy in response to happy faces compared to the CNTR adolescents. Furthermore, the adolescents with CSA-related PTSD reacted significantly slower than the INT adolescents. Although the behavioural results are meaningful and point out interesting group differences, we are not able to interpret them in combination with the results of the fMRI analyses. Future research could solve the limitations by using an optimized task design

specifically developed to investigate habituation effects and its relation with behavioural results. For example by using a passive viewing task and a task focussing on constrained attention that both include positive, negative and neutral emotional faces and have enough trials to examine habituation patterns. Including a passive viewing task would also decrease the influence of attention load on amygdala activation (Costafreda et al., 2008; Sauer et al., 2013). Related to this is the inclusion of pictures of emotional faces from two different databases (Karolinska (Lundqvist et al., 1998) and NimStim faces (Tottenham et al., 2009)) without matching the faces on size and centering. Future research should match the faces on size and centering. However, the faces were randomly presented and therefore it is unlikely that the differences in centering affected the habituation effect. Another limitation is the significant difference in age and IQ between the control group and the CSA-related PTSD group. Although we controlled for age and IQ in the analyses, results might have been influenced by these differences. Future research should include participants within smaller age ranges who are matched on gender and IQ. It would also be interesting to include several age ranges within adolescence to investigate developmental differences between and within groups, since previous research has indicated that there are relatively large developmental changes within the face processing network including the amygdala (Hare et al., 2008; Scherf et al., 2012).

Taken together, this study indicated that adolescents with internalizing disorders showed different patterns of amygdala activation and habituation to emotional faces than adolescents with CSA-related PTSD. These findings inform our understanding of individual differences in adolescence by showing that adolescents with overlapping psychiatric complaints can show different patterns of habituation to emotional face stimuli. Possibly this can provide information for the development of better intervention and treatment strategies: if replicated across samples, the results may indicate that it is potentially more helpful to focus on reducing the primary emotional responses in adolescents with CSA-related PTSD and to focus on top-down regulation in adolescents with internalizing disorders.

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