The Affective Amygdala : towards a better understanding of adolescent depressive and anxiety disorders

Bulk, B.G. van den

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Cover Page

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Habituation to emotional
faces in depressed and
anxious adolescents

Abstract

Adolescents with depressive and anxiety disorders and adolescents who experienced childhood sexual abuse show a large overlap in symptomatology. Research indicated hyper responsiveness and sustained activation instead of habituation of amygdala activation to emotional faces in adolescents with depressive and anxiety disorders and in adolescents who experienced childhood trauma. Little is known, however, about whether the same patterns of amygdala activation and 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 who experienced childhood sexual abuse (CSA; N=19) and healthy controls (N=26). Behaviorally, adolescents with depressive/anxiety disorders and adolescents who experienced CSA reported more anxiety to fearful and neutral faces than controls. On whole brain level, there was a significant interaction between run and group within the left amygdala. ROI analyses showed elevated initial activity in the amygdala and rapid habituation in the CSA group compared to the depression/anxiety and healthy control group. These findings suggest that habituation patterns provide an additional index of emotional face processing problems, possibly showing that fearful responses in trauma groups habituate faster over time, whereas adolescents with depressive and anxiety disorders show less malleability.

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 hundred of milliseconds and provide crucial information for social interactions (Adolphs, 2002; Fusar-Poli et al., 2009; Grossmann, & 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 have reported that activity in this network is restructured in mid adolescence (Casey, Jones, & Somerville, 2011), such that intensified emotion-processing makes the amygdala especially sensitive to reading emotions from faces of unknown others (Scherf, Smyth, & Delgado, 2013). Several studies have reported that the amygdala shows stronger activity to emotional face processing in mid adolescence compared to childhood and adulthood (Guyer et al., 2008; Hare et al., 2008; Pfeifer et al., 2011; Somerville et al., 2011).

At the same time, there are pronounced individual differences in amygdala responsiveness in both adulthood and adolescence. Several reports have shown that responsiveness is higher in individuals who report higher levels of depression or anxiety or are diagnosed with one of these disorders (Monk et al., 2008b; Roberson-Nay et al., 2006; Somerville et al., 2004; Thomas et al., 2001a; Van Den Bulk et al., 2014), or who have a history of childhood maltreatment such as emotional, physical or sexual abuse (Garrett, Carrion, Kletter, Karchemskiy, Weems, & Reiss, 2012; Gee et al., 2013a; Hart, & Rubia, 2012). These findings suggest that reactivity of the amygdala may be more intense in individuals who report emotional problems. Individuals who experienced childhood maltreatment and who develop subsequent Post-Traumatic Stress Disorder (PTSD) are at increased risk to develop depressive and anxiety disorders over the course of life (Lindert, Von Ehrenstein, Gras-

how, Gal, Braehler, & Weisskopf, 2014). This, in combination with the comparable levels of increased amygdala activation in response to emotional faces (Hart, & Rubia, 2012; Monk et al., 2008a; Monk et al., 2008b; Roberson-Nay et al., 2006; Thomas et al., 2001a), highlights the need to investigate whether similar underlying neurobiological mechanisms are present in these groups. To our knowledge, there is no research published that directly compared the underlying higher amygdala responsiveness in adolescents with a depressive and/or anxiety disorder and adolescents who report emotional problems because of experiencing childhood sexual abuse (CSA). It is possible that there are neurobiological differences between these two groups: adolescents who experienced CSA have distinct characteristics like the experience of one or more traumatic events, which might have caused the activation of different underlying neurobiological mechanisms for depression and anxiety related symptoms.

Even though it is challenging to reveal differentiating neurobiological mechanisms between highly related clinical disorders, one way to examine whether the groups have different underlying response patterns is by studying habituation effects. In healthy populations, it is well known that the amygdala habituates to observed emotional expressions over time (Breiter et al., 1996; Fischer, Wright, Whalen, Mcinerney, Shin, & Rauch, 2003). The results of studies investigating habituation of amygdala activation in individuals with inhibited states, like depression and anxiety, are inconsistent. For example, a study by Hare and colleagues (2008) showed that adolescents with higher self-reported anxiety ratings habituated more slowly to observing emotional faces than adolescents with low levels of self-reported anxiety ratings. However, this study did not include information about the cause of heightened self-reported anxiety: it was not known whether it was related to childhood trauma or general patterns of anxiety independent of trauma. Two other studies reported relatively strong habituation effects during face processing within the amygdala in a sample of adults with social anxiety disorder (Sladky et al., 2012) and a sample of female students scoring high on

fear questionnaires (Wendt, Schmidt, Lotze, & Hamm, 2012). Again, no information on childhood trauma was available. To extend the current literature, it is of interest to compare amygdala habituation patterns in adolescents with depressive and/or anxiety disorders and adolescents who experienced childhood maltreatment, such as CSA.

In this study, we examined amygdala habituation in two groups that have previously been found to show elevated amygdala responsiveness to emotional faces. We included individuals with a DSM-IV diagnosis of a depressive or anxiety disorder, adolescents who experienced CSA, and a matched control group of adolescents without psychiatric complaints or traumatic experiences. Participants performed an emotional face-processing task validated in prior work (Monk et al., 2003a; Van Den Bulk et al., 2013; Van Den Bulk et al., 2014), and we reanalyzed the data for habituation patterns for subgroups of individuals by separating the task in three runs.

We aimed to test for dissociable habituation effects between groups based on the hypotheses that healthy control group participants will show fast habituation in the amygdala (Breiter et al., 1996), that both clinical groups will show increased amygdala activation in response to emotional faces (Garrett et al., 2012; Mcclure et al., 2007b; Roberson-Nay et al., 2006) and that the depression/anxiety group will show sustained activation in the amygdala (Hare et al., 2008). We were particularly interested in whether adolescents with CSA showed a similar pattern as adolescents with depression/anxiety without trauma, or whether their neural patterns were dissociable, suggesting that their anxiety and depression symptoms are related to a different underlying neural sensitivity.

Methods
Participants
Functional MRI data were collected based on 31 healthy controls, 30 treatment naïve adolescents with a clinical diagnosis of a current DSM-IV de-

pressive or anxiety disorder but no childhood trauma, and 22 adolescents who experienced childhood sexual abuse (CSA; comorbidity with anxiety and/or depression due to the 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 (> 3mm.; 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 healthy controls, 26 adolescents with a depressive or anxiety disorder and 19 adolescents with CSA (Table 1). All adolescents took part in the larger EPISCA study (Emotional Pathways' Imaging Study in Clinical Adolescents). The two clinical groups were scanned before the start of regular Cognitive Behavioral Therapy (CBT) based treatment.

    Adolescents from the two clinical groups were recruited in outpatient departments of tree child and adolescent psychiatric institutes in Leiden and Haarlem. Inclusion criteria for participants in the depression/anxiety 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 group were: having lifetime experiences of sexual abuse by one or more perpetrators in- or outside the family and being referred for CBT-based therapy. Adolescents in the control group were recruited through local advertisements, with the following inclusion criteria: no clinical scores on validated mood and behavioral 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 contra-indications.

    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<.05) and IQ ($F_{(2,70)}$=3.63, p<.05), but not for sex distribution ($\chi^2_{(2,71)}$=.28, p=.87). The CSA group was significantly older and scored significantly lower on the IQ test than the control group (p<.05 and p<.05). The depression/anxiety group did not significantly differ from the control and CSA group (all p's>.10). For this reason, age and IQ were added as covariates in all subsequent 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 their participation. The Medical Ethics Committee of the Leiden University Medical Centre approved the study and all anatomical scans were reviewed and cleared by a radiologist.

Table 1. Participant characteristics of adolescents with a depressive/anxiety disorder, CSA adolescents and healthy control group adolescents.

	Depr./anx.	CSA	Control	χ^2	df	p	
	N	**N**	**N**				
N	26	19	26				
Females/Males	22/4	17/2	23/3	.282	2	.868	
	Mean$_{(sd)}$	**Mean$_{(sd)}$**	**Mean$_{(sd)}$**	**F**	**df**	**p**	
Age	15.98$_{(1.45)}$	16.62$_{(1.79)}$	15.25$_{(1.64)}$	4.02	2,70	.022	CSA>CNTR
Full scale IQ	105.12$_{(8.66)}$	99.89$_{(9.10)}$	106.58$_{(7.77)}$	3.63	2,70	.032	CNTR>CSA
DSM-IV depression/anxiety classification:	**N$_{(\%)}$**	**N$_{(\%)}$**	**N$_{(\%)}$**				
No depressive/anxiety disorders	0	19$_{(100\%)}$	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				
DSM-IV PTSD classification:							
No PTSD	19$_{(73\%)}$	1$_{(5\%)}$	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				
Self-reported symptomatology[†]	**Mean$_{(sd)}$**	**Mean$_{(sd)}$**	**Mean$_{(sd)}$**	**F$_{(group)}$**	**df**	**p**	
CDI: total score[*]	19.06$_{(9.10)}$	15.92$_{(7.12)}$	4.56$_{(3.40)}$	30.62	2,66	<.001	CLIN>CNTR
RCADS: total score anxiety subscales[**]	31.84$_{(14.16)}$	34.69$_{(14.36)}$	14.85$_{(10.83)}$	15.84	2,65	<.001	CLIN>CNTR
TSCC: total score[***]	42.51$_{(22.67)}$	44.31$_{(21.69)}$	17.63$_{(13.80)}$	12.99	2,64	<.001	CLIN>CNTR

†Univariate ANOVA's for CDI, RCADS anxiety and TSCC were corrected for age and IQ; *=questionnaire data was missing for one participant of the depression/anxiety group and three participants of the CSA group; ***=questionnaire data was missing for three participants of the depression/anxiety group and two participants of the CSA group; ***=questionnaire data was missing for three participant of the depression/anxiety group and three participants of the CSA group; CNTR = control group, CLIN = depression/anxiety and CSA 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.

Clinical Assessment

In addition to the clinical assessment as part of the standard intake/ interview procedures by a child and adolescent psychiatrist, the child and parent versions of the Anxiety Disorders Interview Schedule (ADIS) (Silverman, & Albano, 1996) were used to obtain DSM-IV-based classifications of depressive and anxiety disorders and Post Traumatic Stress Disorder (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 control group, and control participants were excluded if they met the criteria for a DSM-IV diagnosis based on the ADIS-interviews or had (sub)clinical scores on clinical questionnaires.

For clinical questionnaires, expectation maximization was used 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.

Task

All participants performed an emotional face-processing task, 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 ('how afraid are you?', 'how happy are you?' and 'how wide is the nose?') and one unconstrained (passive viewing) state questions. After state presentation, participants viewed 21 pictures expressing a fearful, neutral or happy face (a total of 21 trials per state question; presented in 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). Reaction times and subjective scoring of the different emotional faces (fearful, happy or neutral) were recorded for behavioral analyses. The task used a mixed design and different state questions 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 state questions for 4000 milliseconds followed by a fixation cross with a jittered duration between 500 and 6000 milliseconds. Thereafter, one of the pictures was shown for 3000 milliseconds during which participants provided a rating to the probe question (Figure 1). Trials during which the participants did not respond within 3000 milliseconds (1.91% in total) were not included in the behavioral analyses and included as regressor of no interest in the fMRI analyses.

Since we were interested in habituation effects we modeled the three runs separately for the fMRI data. To be sure that enough trials were present per condition, we collapsed across state questions and only focused on emotional valence of the faces (fearful, happy and neutral), which results in 28 trials per condition per run. In prior studies, we found that amygdala activity was not influenced by state questions (van den Bulk et al., 2013; van den Bulk et al., 2014).
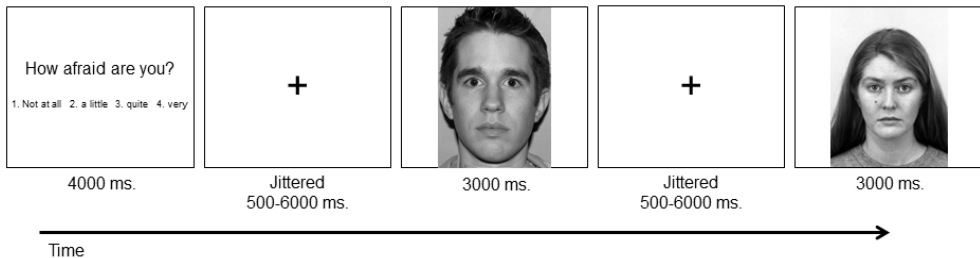

Figure 1. Visual representation of the emotional face-processing task. *Participants were first presented with one of the state questions (i.e., how happy are you, how afraid are you, how wide is the nose or passive viewing) followed by a fixation cross. Thereafter, twenrty-one pictures with a negative,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

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=30ms, flip angle=80°, 80x80 matrix, FOV=220 mm, 38 slices of thickness 2.72 mm) were obtained during three functional runs of 192 volumes each. At the start each run had two additional volumes, 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°; 192x152 matrix; FOV=224x177x168 mm, 140 sagittal slices; no slice gap; 1.16x1.16x1.20 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 were able to indicate their ratings by using a button box, which was attached to their leg.

fMRI analyses

The collected data were analyzed using SPM8 (Welcome 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 3 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, & Tournoux, 1988).

Individual subjects' data were analyzed using the general linear model in SPM8. The fMRI time series were modeled by a series of events convolved with a canonical hemodynamic response function (HRF). The state questions were modeled separately as 4000 millisecond events as covariates

of no interest. The picture presentation of each emotional face was modeled as a zero duration event. In the model, the picture presentation was further divided in nine separate function trials (three runs by three expressed emotions). The modeled 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. 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 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 $p<.05$.

Based on the current literature on face processing and habituation we selected the amygdala as an a priori structure of interest to test our hypotheses on habituation. To analyze voxels within the amygdala we selected Regions Of Interest (ROIs) based on an unbiased contrast of all faces > fixation (N=71; FWE corrected, $p<.05$, at least 10 contiguous voxels), and we constrained the selection of active voxels to be within the anatomical boundaries of the amygdala using MarBaR in SPM8 (http://marsbar.sourceforge.net/; (Brett et al., 2002). This resulted in the right amygdala ROI. The left amygdala ROI was derived from the same contrast but with FDR instead of FWE correction, because it was not significantly active at this stringent 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 percent signal change values (which were derived from the beta values) of the two ROIs were further analyzed using 3 (runs) x 3 (emotions) repeated measurement ANOVAs in SPSS 19 and all post-hoc comparisons were Bonferroni corrected.

Results

Behavioral data

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<.001) in which the depression/anxiety group and the CSA group scored significantly higher than the control group (both p's<.001). 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<.001 and $F_{(2,64)}$=12.99, p<.001 respectively) in which the depression/anxiety group and the CSA group scored significantly higher than the control group (all p's<.001). On all scales, the depression/anxiety and CSA group did not differ from each other.

Subjective rating of emotional faces

For the subjective scoring of emotional faces three separate analyses with run (1-3) and emotion (fearful, happy, neutral) as within-subject factors and group as a between subjects factor were performed using repeated measurement ANOVAs in SPSS 19. The scores were analyzed separately for each state question, because values of the scores represent different interpretations for each question. In case sphericity could not be assumed, a Greenhouse-Geisser correction (GG-corr.) was used. Post-hoc comparisons were Bonferroni corrected.

The repeated measurement ANOVA for the state 'how afraid are you?' resulted in a main effect of group ($F_{(2,64)}$=4.19, p<.05) and an emotion x group interaction effect ($F_{(4,128)}$=3.29, p<.05). This interaction revealed that the adolescents with a depressive/anxiety disorder (p<.05) and the adolescents with CSA (p<.05) gave higher scores to fearful faces than the control adolescents. For happy and neutral faces there were no significant differences between groups (all p's>.10; see Figure 2).

The ANOVA for the state 'how happy are you?' resulted in a main effect of group ($F_{(2,62)}$=5.56, p<.01), but no group x emotion interaction. The

main effect of group showed that the overall subjective scoring of the control group was higher than for the depression/anxiety group (*p*<.01), whereas the CSA group did not differ from the anxiety/depression or the control group (both *p*'s>.15).

Finally, the ANOVA for the state 'how wide is the nose?' resulted in a main effect of emotion ($F_{(2,130)}$=5.98, *p*<.005), with higher subjective scoring for happy and fearful faces compared to neutral faces (both *p*'s<.001), and higher subjective scoring for happy than for fearful faces (*p*<.001). There was no main/interaction effect with group.

There was no main or interaction effect of run in any of the state questions suggesting an absence of habituation at the behavioral level.
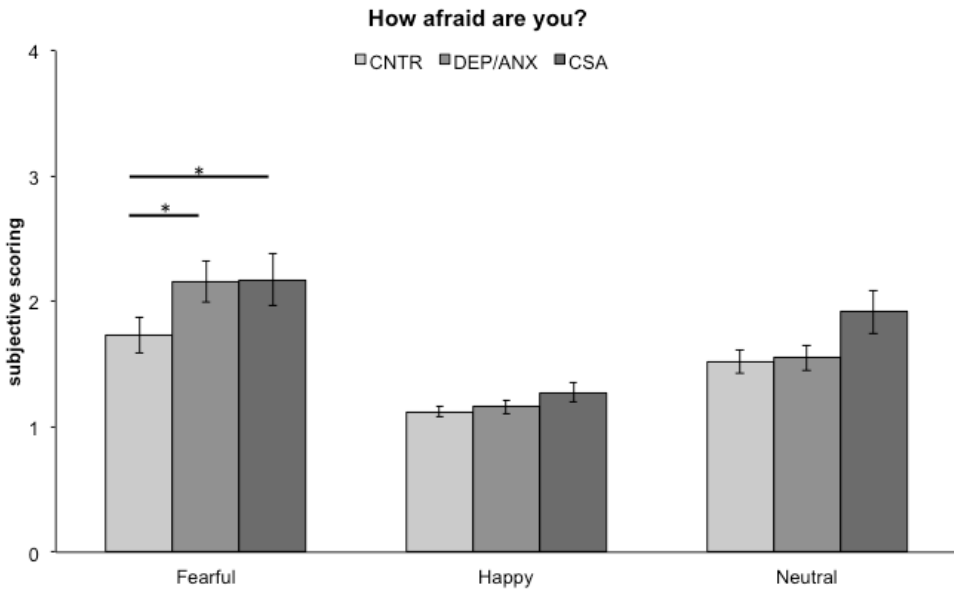

Figure 2. Group differences in subjective scoring of emotional faces with the 'How afraid are your?' attention state. *The two clinical groups reported being more afraid for fearful faces than the control group. * p < 0.05; CNTR=control group; DEP/ANX=depressed anx anxious adolescents; CSA=adolescents who experienced childhood sexual abuse.*

Whole brain analyses

The whole brain analysis for all faces > fixation resulted in robust activation in right amygdala and bilateral insula across participants (Figure 3A). The contrast run 1>run 3 resulted in significant activation in bilateral amygdala, suggesting changes in amygdala activation over time across participants. To follow-up the run effect, we inspected the main effect of group within the contrast run 1>run 3 (i.e., a group x time interaction). The results showed a significant group effect specifically in the left amygdala (uncorrected, p<.001, 10 voxels, no regions were detected when applying FDR or FWE correction; Figure 3B). Follow up t-tests for the contrast run 1>run 3 for each group separately revealed activation in this region only for the CSA group (p<.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.

Region of interest analyses

Region of interest analyses were performed for the right and left amygdala in a run x emotion x group repeated measurement ANOVA. For right amygdala (Figure 4), the repeated measurement ANOVA resulted in a run x group interaction effect ($F_{(4,132)}$=2.62, p<.05). For the CSA group there was a significant decrease in activation between run 1 and run 2 (p<.05), between run 1 and run 3 (p<.001) and between run 2 and run 3 (p<.01). A comparable pattern of a decrease in amygdala activation was seen for the control group: run 1-run 2, p<.05 and run 1-run 3, p<.01. For the depression/anxiety group there were no significant in- or decreases in activation over runs ($p's$>.10). Furthermore, the CSA group showed significantly more amygdala activation in run 1 compared to the depression/anxiety group (p=.05). The three groups showed no significant differences in run 2 and run 3 (all $p's$>.10).
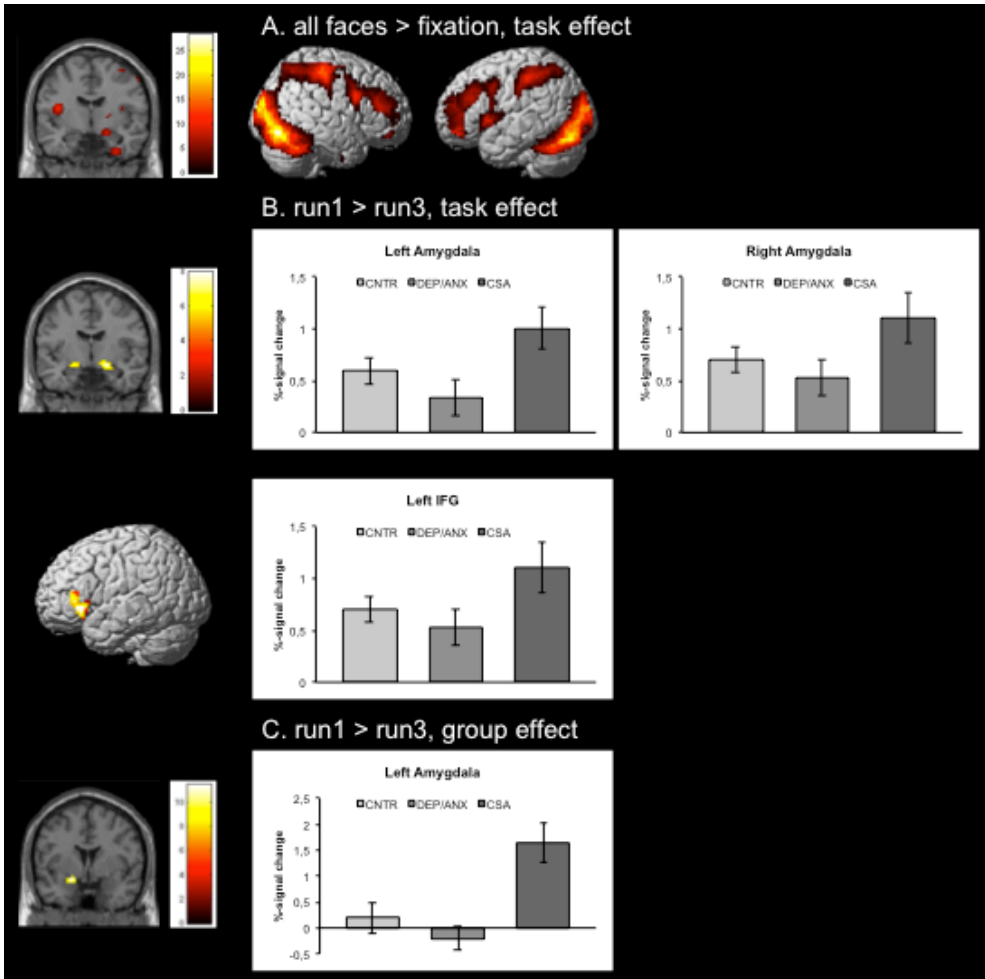
Figure 3. Overview of whole brain results derived from a full factorial model including three groups and three runs. *A. the contrast all emotional faces > fixation for the main effect of the task (FWE corrected, p<.05; 10 contiguous voxels), B. the contrast emotional faces in run 1 > emotional faces in run3 for the main effect of the task (FWE corrected, p<.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<.001; 10 contiguous voxels). Left and right amygdala and left inferior frontal cortex (represented in B and C) were followed up by ROI analyses to visualize the direction of the effects. CNTR=control group; DEP/ANX=depressed anx anxious adolescents; CSA=adolescents who experienced childhood sexual abuse.*

The same analysis for the left amygdala also resulted in a run x group interaction ($F_{(4,130)}$=3.85, *p*=.005). The CSA group showed a significant decrease in activation between run 1 and run 2 (*p*=.001) and between run 1 and run 3 (*p*<.001). For the control group there was a significant decrease in acti-
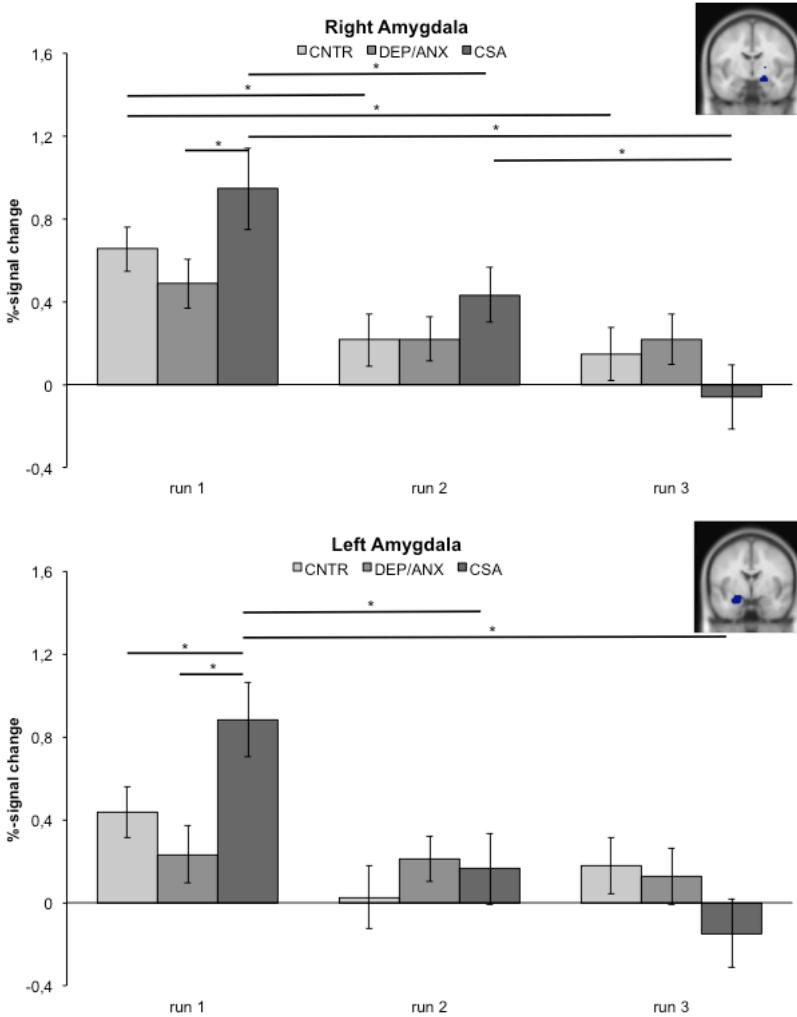

Figure 4. Region of interest analyses for left and right amygdala. *Regions were derived from the contrast all emotional faces > fixation with a FEW correction for right amygdala (p<.05; 10 contiguous voxels) and a FDR correction for left amygdala p<.05; 10 contiguous voxels).   * p < 0.05; CNTR=control group; DEP/ ANX=depressed anx anxious adolescents; CSA=adolescents who experienced childhood sexual abuse.*

vation between run 1 and run 2 (p=.05). Again, within the depression/anxiety group there was no habituation effect ($p's$=1.00). Also, the CSA group showed significantly more activation in run 1 compared to both the depression/anxiety group (p=.001) and the control group (p<.05). There was no significant difference between the depression/anxiety group and the control group in run 1 and the three groups did not significantly differ from each other in run 2 and run 3 (all $p's$>.10).

To summarize, the results for both right and left amygdala showed elevated initial activity and rapid habituation of the amygdala in the CSA group when compared to the depression/anxiety group in which no habituation was detected. Overall, no significant main/interaction effects were found for facial expression (all $p's$>.10), suggesting that these effects were consistent across facial expressions.

Discussion

The goal of this study was to examine whether amygdala habituation during an emotional face-processing task differed between adolescents with a DSM-IV diagnosis of depression and/or anxiety disorder, adolescents who experienced CSA and healthy controls. This is important since depressed/anxious adolescents and adolescents with CSA not only show a large overlap in symptomatology (Lindert et al., 2014), but they also show distinct characteristics: adolescents who experienced CSA per definition experienced one or more traumatic events that might have influenced the development of different neurobiological mechanisms.

Consistent with prior studies (Breiter et al., 1996; Fischer et al., 2003), healthy adolescents showed a habituation effect in the amygdala (especially right) when viewing emotional faces: activation in right and left amygdala was significantly higher during run 1 than during run 2/run 3. This effect was present for all emotional faces, so not solely for fearful faces, which is in line with results of previous studies (Breiter et al., 1996). This suggests that habi-

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

Within the clinical groups, habituation-related amygdala activity showed different patterns. For the CSA group we found initial increased activation in the amygdala and relatively fast habituation of amygdala activation to a level comparable to that of the depression/anxiety and control groups. In the depression/anxiety group we did not find significant habituation effects in the amygdala. Instead, the adolescents with depressive and/or anxiety disorders showed comparable levels of amygdala activation as the control group but showed no significant decline in amygdala activation over the three runs. The analyses partially confirmed our hypothesis: the control group showed a habituation effect in the amygdala while the depression/anxiety group did not show this effect. In addition, the results showed a difference between the two clinical groups in amygdala activation in which the CSA group had a higher initial response to emotional faces at the start of the task and showed faster habituation compared to the depression/anxiety group.

Contrary to prior reports (Mcclure et al., 2007b; Monk et al., 2008a; Monk et al., 2008b; Thomas et al., 2001a), the depression/anxiety group did not show a general higher amygdala response to emotional faces than the healthy 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 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 left to examine habituation during passive viewing. Furthermore, passive viewing was preceded and followed-up by the other conditions and it is not clear to what extend attention load effects continue to be present. Future research should further investigate this by, for example, using a passive viewing task with a sufficient number of trials per run. Group differences may then be more pronounced.

The innovative aspect of the current study was that we included both adolescents with depressive/anxiety disorders and adolescents who experienced CSA. Although the overlap in reported symptomatology between the two clinical groups is high, CSA has an additional component namely the experience of one or more traumatic events. Previous research has indicated that people who experienced childhood maltreatment show heightened patterns of amygdala activation (Hart, & Rubia, 2012; Van Harmelen et al., 2013) and that experiencing childhood maltreatment often leads to the development of depressive and/or anxiety disorders, including PTSD (Lindert et al., 2014). With respect to the behavioral 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, at neurobiological level adolescents with CSA showed higher amygdala activation compared to healthy and depressed/anxious adolescents at the beginning of the task, but similar activation as controls near the end of the task. Possibly, the absence of habituation effects at behavioral level points at a discrepancy between what an individual feels and what is happing in the brain. This possibly relates to the theory of sustained fear levels not being effectively regulated by cognitive control regions (e.g. top-down regulation by the medial prefrontal cortex (PFC) (Mayberg, 1997).

Although speculative, there is a potential interpretation for the different habituation effects between depressed/anxious adolescents and adolescents with CSA. It might be that the depression and anxiety symptoms

reported by adolescents with CSA correspond with increased vigilance to emotional stimuli, which may result in increased amygdala activation in response to emotional faces. However, the down-regulation of this heightened amygdala response might be intact, resulting in habituation over time. In depressed and anxious adolescents a different mechanism might underlie their symptomatology: the primary emotional response is less exaggerated and maybe the integration of information by cognitive control regions is insufficient causing emotion regulation problems. More research is necessary to support this suggestion, for example by using two different paradigms (passive viewing task and emotion regulation task) and by conducting functional connectivity analyses. Within the current task design, it was not possible to conduct functional connectivity analyses because of the relatively fast event-related design and the many conditions.

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 state questions within the emotional face-processing task to have enough power left for the habituation analyses. This limits the ability to isolate specific task effects and possibly suppressed current findings. Future research could optimize this by using a task design specifically developed to investigate habituation effects in the brain. For example, by using a 'pure' passive viewing task including positive and negative emotional faces in which participants only have to indicate the gender of the actor expressing the emotion. This would decrease the influence of attention load on amygdala activation (Costafreda et al., 2008; Sauer et al., 2013). Another limitation is the significant difference in age and IQ between the control group and the CSA group. Although we controlled for age and IQ in all 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 rela-

tively large developmental changes within the face processing network which includes the amygdala (Hare et al., 2008; Scherf et al., 2012).

Taken together, this study indicated that depressed/anxious adolescents showed different patterns of amygdala activation and habituation to emotional faces than adolescents with CSA. These findings inform our understanding of individual differences in adolescence by showing that adolescents with similar symptomatology but with different diagnosis can also show different patterns of habituation to emotional face stimuli. Possibly this can be helpful to improve 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 CSA and to focus on top-down regulation in depressed and anxious adolescents.