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**CHAPTER 5: ENHANCED AMYGDALA
REACTIVITY IN ADULTS REPORTING
CHILDHOOD EMOTIONAL MALTREATMENT**

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ABSTRACT

In the context of chronic childhood emotional maltreatment (CEM; emotional abuse and/or neglect), adequately responding to facial expressions is an important skill. Over time, however, this adaptive response may lead to a persistent vigilance for emotional facial expressions. The amygdala and the medial prefrontal cortex (mPFC) are key regions in face processing. However, the neurobiological correlates of face processing in adults reporting CEM are yet unknown. We examined amygdala and mPFC reactivity to emotional faces (Angry, Fearful, Sad, Happy, Neutral) versus scrambled faces in healthy controls and unmedicated patients with depression and/or anxiety disorders reporting CEM before the age of 16 (n=60), and controls and patients who report no childhood abuse (n=75). We found that CEM is associated with enhanced bilateral amygdala reactivity to emotional facial expressions in general, independent of psychiatric status. Furthermore, we found no support for differential mPFC functioning, suggesting that amygdala hyperresponsivity to emotional facial perception in adults reporting CEM may be independent from top-down influences of the mPFC. These findings may be vital in understanding the increased emotional sensitivity, and interpersonal difficulties, that has been reported in individuals with a history of CEM.

INTRODUCTION

Childhood Emotional maltreatment (CEM) encompasses any act of commission (i.e. verbal abuse) or omission (i.e. emotional neglect) that is (potentially) harmful, or insensitive to the child's emotional development (Egeland, 2009; Gilbert et al., 2009). One in ten children growing up in Western societies experiences CEM per year (Gilbert et al. 2009), and CEM has been associated with a cascade of negative outcomes on behavioral, emotional, and social functioning (Egeland, 2009; Gilbert et al., 2009; Gibb et al., 2007; Spinhoven et al. 2010; Teicher et al. 2006). For instance, CEM is associated with enhanced emotional sensitivity in adulthood, as evidenced by enhanced negative self-associations, depressive symptoms, and difficulties in interpersonal relationships (Egeland, 2009; Gilbert et al., 2009; Spertus et al., 2003; van Harmelen et al., 2010).

In the context of chronic CEM, adequately responding to facial expressions is an important skill. Detecting when a parent is in a bad mood may help a child to avoid a negative confrontation with that parent. However, over time, this adaptive response may lead to a persistent vigilance for negative facial expressions (Gibb et al., 2009). The amygdala is a key brain region involved in the primary processing of emotional faces, and plays a crucial role in salience detection, fear conditioning and emotional memory (Bremner et al., 2005; Davis & Whalen, 2001; Todorov & Engell, 2008; Kim et al., 2011; Onur et al., 2009). In addition, adverse rearing environments in animals, such as maternal isolation, loss, or isolation rearing induce a cascade of long-term alterations on a behavioral and neurobiological level, with specific effects in the amygdala (Sanchez, 2001; McEwen et al., 2012). For instance, maternal deprivation is associated with a lasting enhancement of contextual and cued fear conditioning (Oomen et al., 2010) and anxious behaviour in rats (Eiland & McEwen, 2012).

In humans, acute stress is associated with subsequent amygdala hypervigilance to emotional stimuli (van Marle et al., 2010; Oei et al., 2012). In line with the findings of persistent vigilance in animals, greater left amygdala activation during the processing of negative emotional faces was observed in a small sample of youths who experienced severe emotional and physical neglect in foster care or orphanages (Maheu et al., 2010), and in young adults reporting high childhood family stress (including CEM and physical abuse) while classifying the emotion of fearful and angry faces (Taylor et al., 2006). Thus far, however, it is unknown whether CEM in isolation (i.e. without concomitant physical or sexual abuse) is related to enhanced amygdala activation for negative emotional faces. Furthermore, it is unknown whether CEM related amygdala activation persists into adulthood, i.e., whether adults reporting CEM are characterized by enhanced amygdala response to negative emotional faces.

Using functional-Magnetic Resonance Imaging (f-MRI), an emotional faces task (employing Angry, Fearful, Sad, Happy, Neutral and Scrambled faces) and applying a hypothesis driven Region of Interest (ROI) analysis approach, we examined amygdala functioning in a large sample (N = 135) of

unmedicated outpatients (with depression and/or anxiety) and healthy controls. We investigated whether patients and controls reporting CEM (n=60) showed enhanced amygdala reactivity to emotional faces compared to patients and controls reporting No Abuse (n=75). In line with Maheu et al. (2010), we expected that individuals reporting CEM showed enhanced amygdala response to negative emotional faces (i.e., Angry, Fearful and Sad), but not to happy, or neutral faces. In addition, our group recently reported reduced mPFC gray matter volume in adults reporting CEM (van Harmelen, van Tol et al., 2010). However, it is unknown whether the reduced mPFC volume in these adults reporting CEM (van Harmelen, van Tol et al., 2010) is also related to altered mPFC responsivity. Therefore, we also investigated differences between the CEM and control group with respect to mPFC reactivity to emotional faces.

In addition, to examine whether altered Amygdala and mPFC activation in response to negative emotional faces is related to psychopathology, we investigated whether abnormal amygdala (and/or mPFC functioning) was more apparent in emotionally maltreated individuals with a depression and/or anxiety disorder compared to emotionally maltreated individuals who never developed a depression and/or anxiety disorder.

METHODS

PARTICIPANTS FOR THE NESDA-MRI STUDY

Participants were drawn from the Netherlands Study of Depression and Anxiety (NESDA, N=2981). A subset of this large observational cohort study (Penninx et al., 2008), were selected to undergo Magnetic Resonance Imaging (MRI) scanning (233 patients and 68 HC). All participants underwent MRI in one of the three participating centers (i.e. Leiden University Medical Center, Academic Medical Center Amsterdam, and University Medical Center Groningen). The Ethical Review Boards of each center approved this study. After complete description of the study to the subjects, written consent was obtained.

Inclusion criteria for the NESDA-MRI patients were: current major depressive disorder (MDD) and/or anxiety disorder (AD) (panic and/or social anxiety disorder) in the last 6 months according to DSM-IV criteria. Diagnoses were established using the structured Composite International Diagnostic Interview (Wittchen et al., 1991), administered by a trained interviewer.

Exclusion criteria for patients in the NESDA-MRI study were: the presence of axis-I disorders other than MDD, panic disorder or social anxiety disorder (except generalized anxiety disorder); any use of psychotropic medication other than a stable use of Selective Serotonin Reuptake Inhibitors (SSRI) or infrequent benzodiazepine use (3×2 tablets weekly, or within 48 hrs prior to scanning), the presence of major internal or neurological disorders; dependence or past year abuse of alcohol and/or drugs; hypertension (>180/130mm Hg); heavy smoking (>5 cigarettes/day); and general MRI-

contra-indications. Controls had no lifetime depressive or anxiety disorders and were not taking any psychotropic drugs.

In the current study, some additional exclusion criteria were formulated; patients using SSRI (n=79) were excluded given its potential effect on face processing (Fu et al., 2004; Sheline et al., 2001). Moreover, we excluded 58 participants because of incomplete fMRI data (n=9), technical difficulties (n=24), or poor imaging quality (n=25). See supplement for additional information on the excluded groups.

Table 1. Clinical and demographic characteristics of participants reporting Childhood Emotional Maltreatment vs. No Abuse.

		No Abuse (n=75)	CEM (n=60)	F	χ^2	P
Gender (n)	Male/Female	26/49	20/40		.03	.87
Handedness (n)	Left/Right	9/66	4/56		1.09	.30
Scan location (n)	AMC/LUMC/UMCG	16/32/27	14/28/18		.54	.76
		Mean (SE)	Mean (SE)			
Age		34.91 (1.22)	38.22 (1.32)	3.36		.07
Education		13.32 (0.34)	12.52 (0.38)	2.49		.12
Neuroticism		32.69 (1.26)	39.53 (0.93)	14.83		.00
Recent Life events		0.58 (0.11)	0.83 (0.16)	1.88		.17
Current diagnosis					8.73	.03
Major Depressive Disorder (n)		18	14			
Anxiety Disorder (n)		17	17			
Comorbid Depression and Anxiety Disorder (n)		11	18			
Healthy Controls (n)		29	11			
Lifetime diagnosis						
Major Depressive Disorder (n)		34	44	10.71		.001
Anxiety Disorder (n)		32	38	5.70		.013
Beck Anxiety Inventory		9.19 (1.22)	11.38 (1.11)	1.69		.19
Montgomery Åsberg Depression Rating Scale		7.64 (1.13)	12.88 (1.32)	9.18		.003
Concurrent abuse						
Physical abuse (n)		0	7			
Sexual abuse (n)		0	11			
Physical & sexual abuse (n)		0	5			

Note. CEM= Childhood Emotional Maltreatment, AMC= Amsterdam Medical Center, LUMC= Leiden University Medical Center, UMCG= University Medical Center Groningen.

Childhood Emotional Maltreatment was defined as emotional neglect and/or psychological abuse before the age of 16 years, as measured with the NEMESIS trauma interview (see below for more details concerning this interview). Because we did not expect that a single incident of CEM would chronically alter brain functioning, we excluded individuals reporting only a single incident of CEM (n=24). Moreover, also individuals reporting childhood physical or sexual abuse without CEM (n=5) were excluded. The final sample consisted of 135 participants (see Table 1 for all demographic and clinical characteristics). Individuals who reported emotional neglect or emotional abuse in childhood that had occurred more than once were included in the CEM group (n=60; 30 individuals reported only emotional neglect, two reported only emotional abuse, and 28 reported both emotional neglect and abuse; 59 individuals reported their biological parents as perpetrators, and one subject reported a sibling as the perpetrator). Individuals who did not report emotional, physical, and sexual maltreatment were included in the No Abuse group (n=75; Table 1).

CHILDHOOD EMOTIONAL MALTREATMENT

The CEM and No Abuse groups did not differ in years of education, recent life events, anxious symptomatology, gender, handedness, or scan location (see Table 1). However, the CEM group was slightly older, had higher neuroticism scores, reported more depressive symptomatology, and consisted of more individuals with a current psychiatric diagnosis.

CHILDHOOD MALTREATMENT

Childhood maltreatment was assessed through the NEMESIS trauma interview (de Graaf et al., 2002; 2004). In this interview, respondents were asked whether they had experienced emotional neglect, emotional abuse, physical abuse, and/or sexual abuse before the age of 16, how often this had occurred (never, once, sometimes, regularly, often, or very often), and what their relationship with the perpetrator was. Emotional neglect was described as: *'people at home didn't listen to you, your problems were ignored, you felt unable to find any attention or support from the people in your house'*. Emotional abuse was described as: *'you were cursed at, unjustly punished, your brothers and sisters were favored – but no bodily harm was done'*.

Our definition of Childhood Emotional Maltreatment (i.e. emotional neglect and/or psychological abuse before the age of 16 years) is based on the American Professional Society on the Abuse of Children (APSAC; Bingelli, Hart, & Brassard, 2001; Egeland, 2009). This definition states that emotional child maltreatment consists of acts of commission (emotional abuse such as degrading, terrorizing, belittling, blaming, exploiting) and/or omission (emotional neglect e.g. isolation, rejection, denying emotional responsiveness), that conveys to the child that that he/she is worthless, unloved, and unwanted, and are harmful to the child's emotional developmental needs.

ADDITIONAL QUESTIONNAIRES

Recent life events (past year) were assessed using the List of Threatening Events Questionnaire (Brugha et al., 1985). Neuroticism was measured with the NEO Five-Factor Inventory (Costa & McGrae, 1992). Furthermore, at the day of scanning, depression and anxiety severity (past week) was measured using the Montgomery Åsberg Depression Rating Scale (Montgomery & Åsberg, 1979) and Beck's Anxiety Inventory (Beck et al., 1988).

THE FACES TASK

The faces paradigm was based on the event-related emotional paradigm used by Wolfensberger and colleagues (2008), that has been found to robustly activate the amygdala. Photographs of angry, fearful, sad, happy, neutral faces, and a control condition (scrambled faces) were presented to all participants. The photographs were selected from the Karolinska Directed Emotional Faces System (Lundqvist et al., 1998), representing standardized facial expressions of emotions expressed by amateur actors. Twenty-four stimuli were selected for each of five facial expressions,

comprising twelve female and twelve male faces. Each particular face was not presented more than four times. The scrambled faces were presented eighty times. The faces task was presented using E-prime software (Psychological Software Tools, Pittsburgh, PA, USA). In order to reduce anticipatory effects, an event-related design was employed. This entailed a pseudo-random presentation of a total of 200 stimuli against a black background. Each photograph was shown on the screen for 2.5 seconds, with an inter-stimulus (black screen) interval varying between 0.5 and 1.5 seconds. The images were projected onto a translucent screen at the end of the scanner bed, visible via a mirror above the participant's head. All participants were instructed to indicate each face's gender by pressing one of two buttons with the index finger of the left or right hand. During the presentation of scrambled faces, participants had to press left or right buttons in conformity with an arrow pointing to the left or to the right. The reaction times were recorded.

MRI ACQUISITION

Imaging data were acquired using Philips 3T MR-systems (Best, The Netherlands) located at the University Medical Centers in Leiden, Amsterdam and Groningen, equipped with a SENSE-8 (Leiden and Groningen) and a SENSE-6 (Amsterdam) channel head coil, respectively. For each subject, echo-planar images were obtained using a T2*-weighted gradient echo sequence (repetition time [TR]=2300 ms, echo time [TE]=30ms [Groningen: TE=28 ms], matrix size: 96×96 [Groningen: 64×64], 35 axial slices [Groningen: 39 slices], interleaved acquisition, 2.29×2.29 mm in-plane resolution [Groningen: 3×3mm], 3mm slice thickness). Echo-planar images were scanned parallel to the anterior-posterior commissure plane. To control for differences in scan sites, we added dummy variables for the different scan centers to all analyses. The faces paradigm was part of a functional scanning session utilizing multiple tasks, the results of other tasks are reported elsewhere.

FUNCTIONAL MAGNETIC RESONANCE IMAGING DATA PREPROCESSING

Functional imaging data were preprocessed and analyzed using Statistical Parametric Mapping (SPM)-5 software implemented in Matlab 7.1.0 (www.mathworks.co.uk). Preprocessing, after extensive quality evaluation of the data, consisted of manually reorienting the functional images to the anterior commissure, slice time correction, image realignment, registration of the T1-scan to the mean echo-planar image, warping to Montreal Neurological Institute (MNI)-space as defined by the SPM5 T1-template, reslicing to 3×3×3mm voxels and spatial smoothing using an 8mm full width half maximum Gaussian kernel. Subject movement (>3mm) resulted in exclusion of the data from further analysis.

FMRI DATA ANALYSIS

FMRI-data were analyzed in the context of the General Linear Model. Haemodynamic responses to each stimulus were modeled with a delta function convolved with a synthetic haemodynamic response function. Low-frequency noise was removed by applying a high-pass filter (cut-off 128s) to the fMRI time-series at each voxel. Statistical parametric maps for each comparison of interest were calculated on a voxel-by-voxel basis.

For each subject, contrasts for Facial expressions (Angry, Fearful, Sad, Happy, Neutral) versus scrambled faces were computed. We then conducted a Facial expressions (Angry, Fearful, Sad, Happy, Neutral) \times Group (CEM vs. No Abuse) second level analysis, to examine the main effect of task in our ROIs (i.e., amygdala and mPFC). We specified dummy variables for the different scan centers and a weighted dummy for psychiatric status as covariates. In the weighted dummy for psychiatric status (with values 0 or 0.43), the value for the patient group ($n=94$) was weighed according to the size of the control group ($41/94=0.43$). In addition, because of findings of age and gender related differences in amygdala response for emotional processing (Iidaka et al., 2002; Wager et al., 2003), we also specified age and gender as covariates. All results are reported in MNI space, and significance threshold for the main effect of task was set at $P<0.05$ Family Wise Error (FWE) corrected for multiple comparisons. To investigate if there were any CEM related activations outside our predefined ROIs, we performed a whole brain analysis with at $P<0.05$ FWE corrected.

For the amygdala, we extracted activations for the main effect of task (F) using the Marsbar ROI Toolbox, (Brett et al., 2002). The binary mask in MNI space of the left and right amygdala was specified using the Wake Forest University (WFU) Pick Atlas software, SPM Toolbox (<http://fmri.wfubmc.edu>).

Because the anatomical region of the mPFC is less well defined than the amygdala, group differences (F) in mPFC activations were examined using a Facial expressions (Angry, Fearful, Sad, Happy, Neutral) \times CEM (CEM vs. No Abuse) voxel-wise ROI analysis, while masking for the main effect of task (F). The ROI mask (see Figure 1a) was based on the anatomical location of both dorsal and ventral mPFC (including the anterior cingulate cortex) in MNI space (using the WFU Pick Atlas toolbox). Dummy variables for the different scan centers, gender, age, and psychiatric status (weighted) were specified as covariates. Significance for group differences was set at $P<0.001$ uncorrected, with spatial extent threshold of 5 contiguous voxels.

Based on our previous findings of mPFC volume reductions in CEM in largely the same cohort (van Harmelen, van Tol et al., 2010), group differences in activation within this region were also examined by extracting mean activation differences per valence type within this locus using a binary mask that was based on our earlier findings ($[x=-11\ y=21\ z=44]$; cluster size 767; see Figure 1b, van Harmelen, van Tol et al., 2010).

Following the extraction of Amygdala and mPFC activations (using MARSBAR) we ran correlation analyses to further investigate whether average Amygdala activations for all facial expressions were related to the average mPFC activations for all facial expressions, within the No Abuse, or CEM group.

SPSS DATA ANALYSIS

Psychometric and performance data were analyzed with SPSS 17. Based on the observed distribution of the data, the appropriate parametric (F) or non-parametric Chi-square [χ^2] test was performed. Because the CEM group was slightly older (Table 1), age was defined as a covariate in all SPSS analyses (however, all results remained the same when removing age as covariate). In addition, to investigate whether the results are dependent on psychiatric status, in all analyses, we added a weighted dummy for psychiatric status as a covariate. To further examine the exact impact of psychiatric status on emotional face processing in individuals reporting CEM, we finally performed an additional Diagnosis (MDD, AD, Co-morbid MDD and AD (CDA), Healthy Controls (HC)) \times CEM (No Abuse, CEM) Analysis of Variance.

Significance was set at $P < 0.05$, two tailed, all tests were Bonferroni-corrected for multiple comparisons.

RESULTS

PERFORMANCE ON FACES TASK

Performance on faces was assessed using a Facial expressions (Angry, Fearful, Sad, Happy, Neutral) \times CEM (CEM vs. No Abuse) Repeated Measures (RM) Analyses of Covariance (ANCOVA) on the reaction times. Individuals reporting CEM had similar reaction times as individuals reporting No Abuse ($F(1,130)=0.002$, $P=0.96$) and there was no CEM \times Facial expressions interaction ($F(4,520)=1.03$, $P=0.39$). There was a main effect of Facial expressions ($F(4,520)=2.41$, $P=0.049$, Cohen's $d(d)=0.27$). Compared to the other faces, participants were faster in labeling the gender of angry faces, all P 's < 0.001 , independent of maltreatment-status ($F(4,520)=1.03$, $P=0.39$). Moreover, age had a main effect ($F(1,130)=6.74$, $P=0.011$, $d=0.45$), with older participants having longer reaction times. Psychiatric status did not affect reaction times ($F(1,130)=0.22$, $P=0.64$).

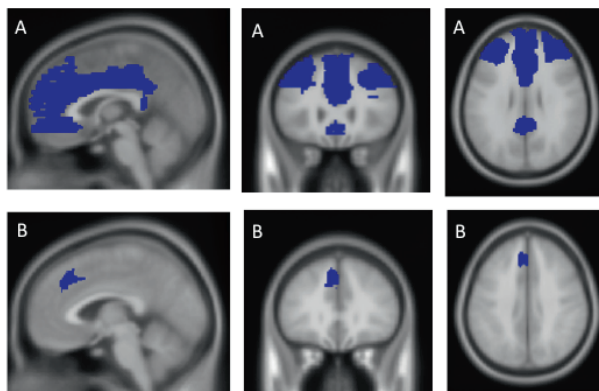
THE MAIN EFFECT OF TASK

A Facial expressions (Angry, Fearful, Sad, Happy, Neutral) \times CEM (CEM vs. No Abuse) analysis showed that the task was associated with significant activations in left and right amygdala ($x=-18$ $y=-6$ $z=-15$, cluster size/number of voxels (K)=236, $Z > 8$, $P < 0.001$) & ($x=18$ $y=-6$ $z=-15$, $K=263$, $Z > 8$, $P < 0.001$) (Figure 2).

Furthermore, the task was also associated with significant activations in the mPFC ($x=6$ $y=36$ $z=24$, $K=50$, $Z=5.52$, $P < 0.001$) & ($x=9$ $y=48$ $z=-3$, $K=37$, $Z=5.04$, $P < 0.001$), all results are FWE corrected. Other regions that were

activated with task (including the right and left Fusiform Gyrus, the middle Occipital Gyrus, and the Superior Frontal Gyrus) are specified in Table 2. However, a Facial expressions (Angry, Fearful, Sad, Happy, Neutral) \times CEM (CEM vs. No Abuse) whole brain analysis at $P < 0.05$ FWE corrected, showed no CEM related activations in any of the task related regions outside our ROIs (see also Table 3).

Figure 1. ROI Masks for the mPFC.



Note. A= Cluster based on anatomical regions of the dorsal mPFC, ventral mPFC and entire ACC. B= Cluster based on structural differences in CEM groups as reported in Van Harmelen, van Tol et al., 2010.

Figure 2. Amygdala activation for the main effect of task (emotional versus scrambled faces).

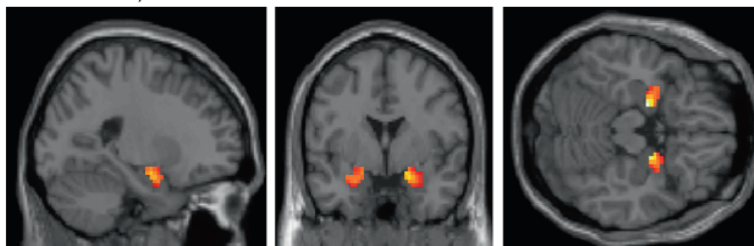


Table 2. Significant gray matter clusters of the main effect of task (F) at $P < .05$ Family Wise Error corrected

	K	F	Z	P	x,y,z
Right Fusiform Gyrus	271	411.86	>8	<.001	42 -51 -21
		145.76	>8	<.001	42 -78 -12
		101.69	>8	<.001	24 -93 -9
Left Fusiform Gyrus	115	200.84	>8	<.001	-39 -51 -21
		198.59	>8	<.001	-27 -90 9
Left Middle Occipital Gyrus	3555	186.01	>8	<.001	30 -87 12
		155.74	>8	<.001	-24 -69 -15
		175.88	>8	<.001	18 -6 -15
Right Amygdala	263	77.15	>8	<.001	27 3 -21
		167.89	>8	<.001	-18 -6 -15
Left Amygdala	236	29.29	5.22	<.001	-39 15 -24
		75.37	>8	<.001	48 24 24
Right Superior Frontal Gyrus	350	74.11	>8	<.001	51 30 18
		64.59	7.75	<.001	45 9 30
		55.29	7.19	<.001	-27 60 9
Left Superior Frontal Gyrus	83	55.29	7.19	<.001	-27 60 9
Right Superior Parietal Lobe	149	53.2	7.05	<.001	54 -48 39
Left Superior Parietal Lobe	375	52.05	6.98	<.001	-51 -48 42
		50.92	6.9	<.001	-45 -48 48
		45.93	6.56	<.001	-60 -39 36
Right Superior Temporal Gyrus	117	44.1	6.43	<.001	66 -21 9
		26.33	4.95	<.001	63 -21 -6
		23.5	4.66	<.001	57 -27 18
Right Superior Frontal Gyrus	11	33.22	5.57	<.001	24 15 54
Right Anterior Cingulate Cortex	54	32.65	5.52	<.001	6 36 24
Right Superior Frontal Gyrus	51	32.33	5.49	<.001	30 54 21
		28.5	5.15	<.001	21 63 6
Right Medial Frontal Gyrus	37	27.36	5.04	<.001	9 48 -3
		26.27	4.94	<.001	3 48 6

Table 3. Significant CEM related gray matter activations outside our ROIs.

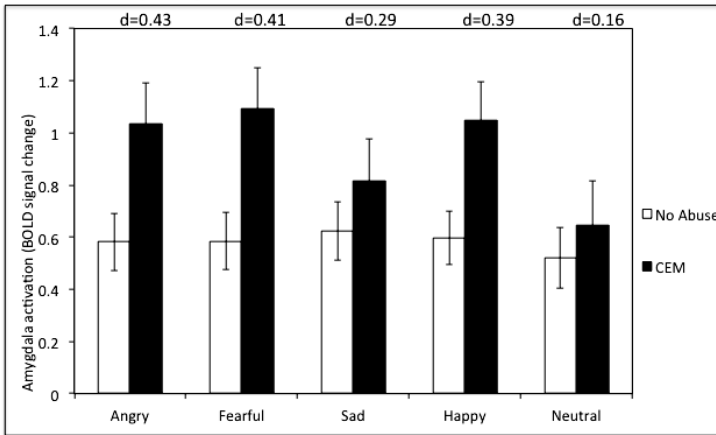
	K	F	Z	P	x,y,z
Left Middle Temporal Gyrus	35	19.52	4.23	<.001	54 -60 -3
Left Lingual Gyrus	21	17.64	4.01	<.001	18 -72 -15
Right Middle Temporal Gyrus	5	14.51	3.61	<.001	36 -81 21
Right Superior Temporal Gyrus	14	13.77	3.51	<.001	51 -30 15
		11.44	3.17	<.001	48 -36 21
Right Lingual Gyrus	9	12.58	3.34	<.001	21 -72 -12
Left Postcentral Gyrus	8	12.55	3.34	<.001	-57 -27 18

Note. Main effect of CEM vs No Abuse (F) whole brain at $P < .001$, uncorrected, with spatial extent threshold of 5 contiguous voxels.

AMYGDALA ACTIVATION IN RESPONSE TO EMOTIONAL FACES

We next extracted bilateral amygdala activations for the main effect of task (F), and conducted a Facial expressions (Angry, Fearful, Sad, Happy, Neutral) \times Lateralization (left amygdala vs. right amygdala) RM ANCOVA with CEM (CEM vs. No Abuse) as fixed factor. Lateralization was added as fixed factor to investigate a possible effect of lateralization (see Maheu et al 2010). In line with our expectations, individuals reporting CEM showed enhanced amygdala activation compared to the No Abuse group ($F(1,131)=5.96$, $P=0.016$, $d=0.43$, see Figure 3).

Figure 3. Estimated marginal means and standard errors of mean amygdala activation in individuals reporting No Abuse vs. Childhood Emotional Maltreatment (CEM).



Note. d = Cohens d for the difference between amygdala activation within the No Abuse versus CEM group.

There was no main effect of Facial expressions ($F(4,524)=0.354$, $P=0.83$), nor Lateralization ($F(1,131)=0.22$, $P=0.64$). Moreover, CEM did not interact with Facial expressions ($F(4,524)=0.89$, $P=0.47$, Lateralization ($F(1,131)=1.25$, $P=0.26$), or Facial expressions \times Lateralization ($F(4,524)=0.89$, $P=0.47$). Finally, age and psychiatric status did not have a main effect on amygdala activation ($F(1,131)=1.45$, $P=0.23$) & $F(1,131)=2.22$, $P=0.14$). Also when performing a Diagnosis (MDD, AD, CDA, HC) \times CEM (No Abuse, CEM) analysis, with Age as covariate, all results remained unchanged, including the main effect of CEM ($F(1,126)=5.26$, $P=0.02$). Moreover, again, diagnosis did not have a main effect on amygdala activation ($F(3,126)=1.28$, $P=0.29$) nor did diagnosis interact with CEM ($F(3,126)=0.22$, $P=0.89$). This absence of a main effect of diagnosis is in line with a recent study in the larger NESDA-MRI sample, where no impact of psychopathology was found on amygdala functioning to emotional and neutral faces (Demenescu et al., 2011).

The CEM group had slightly higher neuroticism, and depression severity scores (Table 1). To investigate whether this could potentially explain our findings, we performed two additional RM ANCOVAs. When we added neuroticism as covariate to the analysis, all results remained unchanged (i.e. main effect of CEM ($F(1,130)=5.98$, $P=0.02$, $d=0.43$)). Neuroticism had no main effect ($F(1,130)=0.23$, $P=0.72$), indicating that the findings cannot be explained by the slightly higher neuroticism scores in the CEM group. Also, when we added severity of depression (as measured with the MADRS) as covariate to the analysis, all results remained unchanged (i.e. main effect of CEM ($F(1,130)=5.18$, $P=0.02$, $d=0.40$)), and depression severity had no main effect ($F(1,130)=1.04$, $P=0.31$), indicating that the findings cannot be explained by higher depression levels in the CEM group. In addition, in both the CEM and No Abuse groups,

Correlation analyses showed no significant relationships between amygdala activations to the emotional and neutral faces and Neuroticism (all P 's > 0.15), nor depression (all P 's > 0.29).

To exclude the possibility that enhanced amygdala reactivity is driven by a concurrent history of physical and/or sexual abuse in some of the participants ($n=23$), we next re-ran the RM ANCOVA while excluding these individuals. In this analysis, all results remained unchanged, including the main effect of CEM ($F(1,108)=5.05$, $P=0.03$, $d=0.39$).

MEDIAL PREFRONTAL CORTEX (MPFC) ACTIVATION IN RESPONSE TO EMOTIONAL FACES

A CEM vs. No abuse (F) ROI analysis (Figure 1a) revealed no significant CEM related activations in the mPFC (ventral and dorsal), nor in the entire ACC. These results remained unchanged when mPFC volume was added as a covariate (van Harmelen, van Tol et al., 2010). Additionally, when we extracted mPFC activations in the mPFC mask that is based on our previous findings (Figure 1b; van Harmelen, van Tol, et al., 2010), a RM ANCOVA showed that CEM did not have a main effect on activation in this region either ($F(1,131)=0.01$, $P=0.91$). Thus, individuals reporting CEM did not differ in mPFC activation in response to emotional facial expressions when compared to individuals reporting No Abuse. Also when performing a Diagnosis (MDD, AD, CDA, HC) × CEM (No Abuse, CEM) analysis, with Age as covariate, all results remained unchanged, including that CEM and diagnosis had no main effect on mPFC activation ($F(1,126)=0.001$, $P=0.98$) & $F(3,126)=1.44$, $P=0.24$), nor was there a CEM × Diagnosis interaction ($F(3,126)=1.29$, $P=0.28$). In addition, no interactions with Facial expressions were found.

CORRELATION BETWEEN AMYGDALA MPFC ACTIVATIONS

Following the extraction of Amygdala and mPFC activations (using MARSBAR) we ran correlation analyses to further investigate whether average Amygdala activations for all facial expressions were related to the average mPFC (Figure 1a) activations for all facial expressions, within the No

Abuse, or CEM group. However, the test yielded no significant relationship between amygdala and mPFC activations in the No abuse group ($r=0.01$, $P=0.92$) nor in the CEM group ($r=0.01$, $P=0.92$). Moreover, a correlational analysis with average amygdala and mPFC activations based on the cluster that we described in van Harmelen, van Tol et al. (2010) (Figure 1b) also yielded no significant relationship in the No abuse ($r=0.06$, $P=0.59$), nor the CEM group ($r=0.14$, $P=0.29$).

DISCUSSION

This is the first study to show that adults reporting CEM show enhanced amygdala activation in response to emotional facial expressions, independent of psychiatric status, neuroticism, depression severity, and history of concurrent physical or sexual abuse. The amygdala plays a key role in detecting the (emotional or biological) salience of stimuli (Kim et al., 2011; Sergerie et al., 2008; Todd & Anderson, 2009; van Wingen et al., 2011), and in enhancing levels of attention and vigilance towards these stimuli (Davis & Whalen, 2001). Therefore, our results suggest amygdalar hypervigilance towards emotional facial expressions in adults reporting CEM. Moreover, together with similar findings in a small sample of adolescents reporting severe neglect (Maheu et al. 2010), our findings suggest sustained hypervigilance even more than 20 years after the maltreatment took place.

Contrary to our hypothesis, hyperactivation of the amygdala in adults reporting CEM was not restricted to negative facial expressions, but was also found in response to happy and neutral faces, although it should be noted that the effect sizes for the neutral faces are relatively small. Therefore, amygdalar hypervigilance to facial expressions in general might indicate that individuals with a history of CEM interpret all facial expressions as highly salient. In line with this idea, neglected children are reported to have poor valence discriminatory abilities for different facial emotions (Pollak et al., 2000; Fries & Pollak, 2004; Vorria et al., 2006), and it has been suggested that neglected children may misinterpret all emotional faces as threatening (Pollak et al., 2000). In that respect, happy faces might be interpreted as a mask for more malevolent emotions (Pollak et al., 2000), for example as being laughed at. Enhanced amygdala activation in response to happy faces could also be indicative of an increased sensitivity towards positive emotional expressions in others (e.g., happy faces might function as safety signal). To disentangle the impact of negative versus positive and neutral faces in individuals reporting CEM, future studies are needed that also assess the subjective ratings of emotional faces besides amygdala activation.

On a neurobiological level, enhanced amygdala responses to all facial expressions may reflect a general noradrenergic sensitization in response to emotional stimuli in individuals reporting CEM. Chronic stress is associated with increased firing of noradrenergic neurons in the brainstem, and augmented release of noradrenalin in the brain following subsequent

stressors (Bremner, Krystal, Southwick & Charney, 1996; Elzinga & Bremner, 2002). Furthermore, enhanced amygdala activation during stress strengthens emotional memory traces and increases fear conditioning (Onur et al., 2009; Joëls & Baram, 2009; Strange & Dolan, 2004). In accordance, in rats, maternal deprivation is associated with a lasting enhancement of contextual and cued fear conditioning (Oomen et al., 2010) and anxious behaviour (Eiland & McEwen, 2012).

Another noteworthy result of this study was that enhanced amygdala reactivity to emotional faces in individuals reporting CEM was observed independent of psychiatric status and that no main effect of diagnosis was observed. This finding is in contrast with other studies finding amygdala hyperreactivity in depressed (Sheline, et al., 2001; Anand et al., 2005; Fales et al., 2008) and anxious patients (Bishop, 2007; Straube, 2004). It should be noted that our patient sample can be characterized as having relatively mild symptoms, due to the fact that we excluded patients using SSRIs. Possibly, this may have lead to an underestimation of the true effect of psychopathology. However, in a larger sample of the same cohort, in which medicated patients were also included, we recently reported no effect of psychiatric status on amygdala reactivity to emotional faces (Demencescu et al., 2011; see for similar findings; Lawrence et al., 2004; Gotlib et al., 2005; Lee et al., 2008; Almeida et al., 2009; Norbury et al., 2009, and a meta-analysis showing no amygdala hyperresponse in depressed patients; Diekhof et al., 2008). Our findings suggest that enhanced amygdala reactivity to emotional faces does not seem to be directly linked to the development of psychopathology in individuals with CEM. Apparently, additional risk factors such as genetic make-up, in itself, or in interaction with exposure to stressful life events during adulthood, or low social support additionally determine who will subsequently develop a depressive and/or anxiety disorder (Hariri et al., 2002; Kilpatrick et al., 2007).

We did not find support for abnormal mPFC functioning in individuals reporting CEM, nor did we find a significant relationship between Amygdala and mPFC activity. Thus smaller mPFC volume (van Harmelen, van Tol et al., 2010) is not related to abnormal mPFC reactivity to emotional facial expressions in adults reporting CEM. Hence, our findings suggests that amygdala hyperresponsivity to emotional facial expressions in individuals with CEM histories may occur independent of the regulatory influences of the mPFC (Fonzo et al., 2000), at least in this gender labeling task which requires minimal cognitive resources (Reddy et al., 2004). However, abnormal mPFC functioning may be observed in tasks posing greater cognitive demands (see for example Shin et al., 2006).

This study is not without its limitations. First, although a clinically diagnosed PTSD diagnosis was an exclusion criterion, unidentified current or lifetime PTSD symptoms may still have been present in the current sample, which may have influenced our findings. This is not very likely, however, given that the enhanced amygdala responses in individuals reporting CEM was unrelated to psychiatric status. Second, history of

childhood maltreatment was retrospectively assessed. In addition, it is important to acknowledge the inherent subjectivity of self-reported CEM. However, it should be noted that, in the current study, neuroticism did not explain enhanced amygdala activation, and in the overall NESDA sample, current affective state did not moderate the association between CEM (as measured with the NEMESIS interview) and lifetime affective disorder (Spinhoven et al. 2010), indicating that recall of CEM in the current sample was not critically affected by current mood state. Furthermore, a recent study showed that depressed women with emotional neglect histories are less prone to produce false memories on the Deese-Roediger, Mcdermott (DRM) task than depressed women with no emotional neglect and women with any type of maltreatment (Grassi-Oliveira et al., 2011). Finally, our findings are based on a cross-sectional design; therefore, one cannot assume causality. It might be that individuals who have strong amygdala reactions to emotional faces may also have experienced certain behaviors of their parents as more abusive or neglectful. Alternatively, enhanced amygdala reactivity to emotional facial expressions may have been pre-existent and inherited by their parents, whose enhanced amygdala reactivity to emotional faces may have increased their risk to emotionally maltreat their children. Theoretically, longitudinal studies are needed to shed more light on the etiology of our findings, although from an ethical point of view, this is problematic. However, recently, a prospective study in soldiers showed that combat stress exposure is associated with enhanced amygdala responsivity to emotional faces, indicative of a causal role of stress exposure on amygdala hypervigilance. In addition, it appeared that the subjective appraisal of threat, and not the actual exposure, played a key role in amygdala regulation in the aftermath of severe stress (van Wingen et al., 2011).

CONCLUSION

We found that adults reporting CEM show enhanced amygdala response to emotional facial expressions. These findings may represent a persistent hypervigilance for emotional facial expressions in adults reporting CEM. Potentially, during social emotional encounters, enhanced amygdala activation in individuals with CEM might result in strong memory traces and increased fear conditioning in response to emotionally significant stimuli (in this case emotional facial expressions). This may be an important key in understanding the increased emotional sensitivity and difficulties in interpersonal relationships (Egeland 2009; Gilbert et al., 2009; Spertus et al., 2003; Teicher et al., 2006; van Harmelen et al., 2010) that has been reported in these individuals.

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SUPPLEMENT

EXCLUSION ANALYSES.

To investigate whether the excluded groups differed systematically on clinical characteristics from our final sample (N=135), we conducted several follow-up analyses of variance, and Chi-square tests. In line with the idea of more severe symptomatology amongst individuals using antidepressants, we found that the SSRI-users had higher neuroticism scores (Mean neuroticism score = 41.95 ± 6.65) than the final sample (Mean neuroticism score = 35.73 ± 10.77) ($F(1, 212)=21.49, p>0.001$). The SSRI-users had more anxious symptomatology (Mean anxious symptomatology = 14.65 ± 8.99) than the final sample (Mean anxious symptomatology = 10.16 ± 9.76) ($F(1, 210)=10.96, P<0.001$). Furthermore, the SSRI-users had more depressive symptomatology (Mean depressive symptomatology = 16.15 ± 9.15) than the final sample (Mean depressive symptomatology = 9.97 ± 10.29) ($F(1, 212)=19.48, P<0.001$). In line, the SSRI-users had more often a psychiatric diagnosis ($X^2=.36.59, df=3, P<0.001$).

With regard to the group of individuals that were excluded because of incomplete fMRI data (n=9), technical difficulties (n=24), or poor imaging quality (n=25), they did not differ from the final sample with regard to anxious symptomatology ($F(1,178)=1.09, P=0.29$), neuroticism score ($F(1, 191)=0.15, P=0.70$) depressive symptomatology ($F(1, 182)=1.48, P=0.22$), nor in number of current psychiatric diagnosis ($X^2=0.07, df=3, P=0.86$).

Finally, the group of individuals (n=29) that were excluded because they reported only a single incident of emotional maltreatment (n=24), or childhood physical or sexual abuse without CEM (n=5) did not differ from the final sample (n=135) in: anxious symptomatology ($F(1,158)=0.89, P=0.35$), neuroticism score ($F(1,162)=0.25, P=0.62$), age ($F(1,158)=0.034, P=0.85$), depressive symptomatology ($F(1,158)=0.09, P=0.77$), and current psychiatric diagnosis ($X^2=1.94, df=3, P=0.58$).