

Interaction of neuropeptide Y genotype and childhood emotional maltreatment on brain activity during emotional processing

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Neuropeptide Y (NPY) has been associated with stress reactivity in affective disorders and is most densely expressed in the amygdala. An important stressor associated with affective disorders is the experience of childhood emotional maltreatment (CEM). We investigated whether the interaction of NPY risk genotype and CEM would affect brain activation. From the Netherlands Study of Depression and Anxiety, 33 healthy controls and 85 patients with affective disorders were scanned with functional magnetic resonance imaging while making gender decisions of emotional facial expressions. Results showed interactions between genotype and CEM, within carriers of the risk genotype, CEM was associated with higher amygdala activation, whereas CEM did not influence activation in non-risk carriers. In the posterior cingulate cortex (PCC), less activation was seen in those with CEM and the risk genotype, whereas genotype did not influence PCC activation in those without CEM. In addition, those carrying the risk genotype and with experience of CEM made a faster gender decision than those without CEM. Thus, the combined effect of carrying NPY risk genotype and a history of CEM affected amygdala and PCC reactivity, areas related to emotion, self-relevance processing and autobiographical memory. These results are consistent with the notion that the combination of risk genotype and CEM may cause hypervigilance.

Keywords: NPY; amygdala; fMRI; depression; childhood abuse

INTRODUCTION

Depression and anxiety disorders are thought to result from maladaptive changes in the stress-response system (Holsboer, 2000). Under conditions of stress, one of many peptides that are released is neuropeptide Y (NPY) (Thorsell *et al.*, 1999). Increased NPY expression is hypothesized to accompany successful behavioral adaptation to stress and may be protective against developing depression or anxiety symptoms (Heilig *et al.*, 2004). This hypothesis is supported by the finding that NPY expression inhibits the release of stress-related hormones, such as adrenocorticotrophic hormone and cortisol (Antonićević *et al.*, 2000). In addition, animal studies have shown that genetically determined low NPY levels are associated with an anxiety-related phenotype during stressful manipulations (Thorsell *et al.*, 2000), which implies a moderating relationship between NPY genotype and experienced stress on affective symptomatology.

In the human gene coding for NPY, the C-allele of the polymorphism rs16147 has been associated with reduced NPY gene expression in

the brain (Zhou *et al.*, 2008; Sommer *et al.*, 2010). Given the evidence from animal studies, the C-allele is likely to predispose to a maladaptive stress response. The C/C-genotype has indeed been related to depression (Heilig, 2004; Mickey *et al.*, 2011) and increased trait anxiety (Zhou *et al.*, 2008). However, other evidence has shown that the association between genotype and depression and anxiety was found only in those C/C-carriers who had also experienced adverse life events (Sommer *et al.*, 2010).

Among stressful life events, childhood emotional maltreatment (CEM) can be considered to be the strongest predictor for developing depressive and anxiety disorders (Hovens *et al.*, 2010; Spinhoven *et al.*, 2010), even more potent than sexual and/or physical abuse (Gibb *et al.*, 2007). This is likely related to the finding that CEM is strongly related to disruptive cognitive styles (e.g. dysfunctional self-attitudes and rumination) (Gibb, 2002; Alloy *et al.*, 2006). For example, experienced CEM has been associated with increased automatic negative self-associations (van Harmelen *et al.*, 2010a,b). In animals, adverse rearing environments such as maternal separation, loss or isolation rearing induce changes at the level of gene expression, hypothalamic–pituitary–adrenal axis functioning, brain morphology and cognitive functioning (Sanchez *et al.*, 2001). Notably, maternal separation has also been shown to reduce NPY levels in animal studies (Jimenez-Vasquez *et al.*, 2001; Husum *et al.*, 2002). Therefore, it could be hypothesized that low NPY expression coded by the C/C-genotype, may interact with the experience of CEM in affecting susceptibility for depressive and anxiety disorders.

In the brain, the highest levels of NPY gene expression have been found in the amygdala (Adrian *et al.*, 1983; Marcos *et al.*, 1999),

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although elevated levels have also been observed in other regions (e.g. anterior cingulate cortex and hippocampus) (Redrobe *et al.*, 1999; Sommer *et al.*, 2010). The amygdala is a key region for identifying the emotional significance of stimuli (Phillips *et al.*, 2003) and in the reaction to stress (van Marle *et al.*, 2009). Amygdala function has been extensively investigated in association with affective disorders, but with conflicting results. Most studies have shown increased amygdala activation in response to emotional stimuli in depressed patients (Matthews *et al.*, 2008; Peluso *et al.*, 2009; Townsend *et al.*, 2010; Victor *et al.*, 2010), but many reported no difference (Irwin *et al.*, 2004; Lawrence *et al.*, 2004; Gotlib *et al.*, 2005; Lee *et al.*, 2007; Friedel *et al.*, 2009; Almeida *et al.*, 2010), including the Netherlands Study of Depression and Anxiety (NESDA), of which this study is part (Demenescu *et al.*, 2011). As NPY is predominantly expressed in the amygdala, amygdala function is likely to be affected by NPY genotype. To date, only a few studies have reported on the association between NPY genotype and emotional processing in the amygdala (Zhou *et al.*, 2008; Domschke *et al.*, 2010). In these studies, healthy people (Zhou *et al.*, 2008) and anxious depressed patients (Domschke *et al.*, 2010) with the C/C-genotype showed hyperactivation of the amygdala in response to negative stimuli. Childhood maltreatment has been associated with heightened amygdala activation in response to emotional stimuli (McCrory *et al.*, 2011; van Harmelen *et al.*, 2013). Recently, a study by our group has shown that specifically CEM was associated with increased amygdala activity in response to emotional faces (van Harmelen *et al.*, 2013). In addition, as stated earlier, life events and NPY genotype may interact with respect to susceptibility for psychopathology. Therefore, it could be hypothesized that NPY genotype and CEM interact on amygdala activation, which has been suggested to be an endophenotype (Savitz and Drevets, 2009), reflecting increased vulnerability for depression and/or anxiety disorders.

The aim of this study was 3-fold. First, to investigate whether NPY genotype influenced amygdala activity (and other brain areas involved in emotion processing); second, whether CEM could influence the effect of NPY genotype on the amygdala and third, because of the possible associations between amygdala activity and affective psychopathology, whether these effects on the brain are different in the presence of affective disorders. We hypothesized that the combined effect of carrying the risk genotype (C/C-carriers) and a history of CEM would be associated with highest amygdala activation.

METHODS

Participants

This study was part of the multi-center NESDA (Penninx *et al.*, 2008), in which University Medical Center Groningen (UMCG), Leiden University Medical Center and VU University Medical Center Amsterdam participated. All participants provided written informed consent and the ethical review boards of each participating center gave approval.

Exclusion criteria for all participants were (i) presence or history of a neurological disorder or somatic disorder with possible effects on the central nervous system, (ii) general magnetic resonance imaging (MRI) contraindications, (iii) dependency or recent abuse (past year) of alcohol or drugs and (iv) hypertension. We chose to only include unmedicated patients in our primary analysis, to exclude a possible confounding effect of medication use on amygdala activity. Diagnosis of major depressive disorder (MDD) and/or anxiety (ANX, social anxiety disorder, panic disorder, generalized anxiety disorder) was based on the Composite International Diagnostic Interview (Andrews and Peters, 1998) administered within 3 months before scanning by specially trained clinical research staff. The sample

consisted of patients with a diagnosis in the past 6 months of MDD, ANX or comorbidity of MDD and ANX (CAD).

For our primary analysis in the unmedicated sample, 118 unrelated Caucasian participants [33 healthy controls (HCs) and 85 patients] were included with complete imaging data (without artifacts) and genotyping (see for demographic details, Table 1). In an additional analysis, we also included patients using selective serotonin inhibitors (SSRIs), to explore possible effects of increasing our sample size. This sample consisted of 165 participants in total (Supplementary Table S1). Participants in this study were all also included in the previously described NESDA-MRI samples of Demenescu *et al.* (2011) and van Harmelen *et al.* (2013). However, this sample is smaller than the previously described samples, because high-quality genotype data for the NPY gene were not available from all subjects.

Genotyping

Genotyping was performed in the context of the genome wide association study of the Genetic Association Information Network (Sullivan *et al.*, 2009). Perlegen Sciences (Mountain View, CA, USA) performed all genotyping according to standard operating procedures. High-density oligonucleotide arrays were used yielding 599 164 single nucleotide polymorphisms (SNPs). On the basis of the linkage disequilibrium structure using the HapMap CEU data (release 22, build 36) as the reference database, the rs16147 SNP, a T- to C-base substitution SNP, could be imputed. The imputation was performed by IMPUTE version 0.3.2 using the default settings and the recommended number 11 418 for the effective population size of Caucasians (Marchini *et al.*, 2007). The quality of the imputation was good (SNPTEST proper_info = 0.99).

Clinical assessments

Depression severity was determined by the Montgomery-Åsberg Rating Scale (MADRS) (Montgomery and Åsberg, 1979), and anxiety severity was determined by the Beck Anxiety Inventory (BAI) (Beck *et al.*, 1988).

CEM was assessed retrospectively using a semi-structured childhood trauma interview, previously used in the Netherlands Mental Health Survey and Incidence Study (NEMESIS) (de Graaf *et al.*, 2004a,b). In this interview, participants were asked whether they had experienced emotional neglect or psychological abuse before the age of 16 years. CEM was defined as multiple incidents of emotional neglect or psychological abuse. This definition has been used previously in the NESDA sample (van Harmelen *et al.*, 2010a,b, 2013) and is based on the definition provided by the American Professional Society on the Abuse of Children (Binggeli *et al.*, 2001; Egeland, 2009).

Emotional processing

During scanning, participants performed an implicit emotional face processing task, which was described earlier (Demenescu *et al.*, 2011). The experimental paradigm was presented using E-prime software (Psychological Software Tools, USA). Photographs with neutral or emotional facial expressions (angry, fearful, happy and sad) from a widely used set (Lundqvist *et al.*, 1998) were shown. The facial expressions were expressed by amateur actors. Twenty-four stimuli were selected for each of five facial expressions, comprising 12 female and 12 male faces. Each face was not presented more than four times. Participants were asked to judge the gender of the person on the photograph and indicate this with a button press. As a baseline condition, 80 scrambled faces with an arrow ('<<' or '>>') were shown indicating which button to press. To reduce anticipatory effects, an event-related design was used that involved a pseudo-random presentation of a total of 200 stimuli against a black background. Each

Table 1 Demographic and clinical characteristics of the participants included

	Risk genotype	Non-risk genotype	Test	<i>P</i>
Cases, <i>N</i> (%)	38 (32.2)	80 (67.8)		
CEM, <i>N</i> (%)	10 (26.3)	46 (57.5)	$\chi^2(1) = 10.05$	0.002
Gender, number of females (%)	26 (68.4)	54 (67.5)	$\chi^2(1) = 0.10$	0.92
Age, mean (s.d.)	39.79 (10.27)	36.54 (9.96)	$t(116) = 1.64$	0.10
Education (in years), mean (s.d.)	13.21 (3.58)	12.76 (3.02)	$t(116) = 0.71$	0.48
Diagnosis, HC/MDD/ANX/CAD	14/12/5/7	19/21/16/24	$\chi^2(3) = 3.83$	0.28
MADRS, mean (s.d.)	9.03 (9.27)	11.50 (10.49)	$t(116) = 1.24$	0.22
BAI, mean (s.d.)	7.97 (8.93)	10.66 (9.64)	$t(116) = 1.45$	0.15
RTs (in ms)				
Angry, mean (s.d.)	809.83 (162.93)	851.35 (166.24)		
Fear, mean (s.d.)	860.27 (180.07)	885.93 (176.87)		
Sad, mean (s.d.)	857.76 (161.28)	887.72 (164.71)		
Happy, mean (s.d.)	873.06 (166.95)	891.32 (161.91)		
Neutral, mean (s.d.)	855.06 (158.56)	892.39 (172.36)		
	CEM	No CEM	Test	<i>P</i>
Cases, <i>N</i> (%)	56 (47.5)	62 (52.5)		
Genotype, number of risk genotype	10 (17.9)	28 (45.2)	$\chi^2(1) = 10.05$	0.002
Gender, number of females (%)	40 (71.4)	40 (64.5)	$\chi^2(1) = 0.64$	0.42
Age, mean (s.d.)	37.86 (9.59)	37.34 (10.67)	$t(116) = 0.27$	0.78
Education (in years), mean (s.d.)	12.52 (3.08)	13.26 (3.30)	$t(116) = 1.28$	0.21
Diagnosis, HC/MDD/ANX/CAD	9/13/14/20	24/20/7/11	$\chi^2(3) = 12.98$	0.005
MADRS, mean (s.d.)	13.25 (10.22)	8.40 (9.57)	$t(116) = 2.66$	0.009
BAI, mean (s.d.)	11.86 (8.59)	7.94 (9.89)	$t(116) = 2.28$	0.02
RTs				
Angry, mean (s.d.)	837.98 (175.05)	837.97 (158.08)		
Fear, mean (s.d.)	875.66 (176.85)	879.47 (179.60)		
Sad, mean (s.d.)	891.13 (179.51)	866.28 (148.11)		
Happy, mean (s.d.)	884.64 (169.91)	886.57 (157.97)		
Neutral, mean (s.d.)	880.51 (172.33)	880.25 (165.94)		

The table shows the demographic and clinical data divided by genotype and the same data, but divided according to CEM.

photograph/picture was shown for 2.5 s, with an interstimulus (black screen) interval varying between 0.5 and 1.5 s. Responses and reaction times (RTs) were recorded.

Image acquisition

All participants were scanned using a Philips 3-T MR-scanner. A sense-8 channel head coil was used for radio frequency transmission and reception. In Amsterdam, a sense-6 channel head coil was used.

A series of echo planar imaging (EPI) volumes was obtained, entailing a T2*-weighted gradient-echo sequence using axial whole-brain acquisition, with an interleaved slice acquisition order and with the following settings: repetition time (TR) = 2300 ms, echo time (TE) = 28.0 ms in Groningen and 30 ms in Amsterdam and Leiden and a flip angle of 90°. At UMCG, 39 slices per EPI volume were acquired, with a matrix size of 64 × 64 voxels and an in-plane resolution of 3 × 3 mm. In Amsterdam and Leiden, 35 slices per EPI volume were acquired, with a matrix size of 96 × 96 voxels and an in-plane resolution of 2.29 × 2.29 mm. The slices had a 0-mm gap and 3-mm thickness. The images were acquired parallel to the anterior–posterior commissure plane.

In addition, a T1-weighted anatomical MRI scan was obtained (TR = 9 ms, TE = 3.5 ms, matrix size 256 × 256 and voxel size: 1 × 1 × 1 mm).

Statistical analyses

Genotype data

To test if the genotype distribution was in Hardy–Weinberg equilibrium (HWE), a chi-square test was performed.

Clinical and behavioral data

To test for effects of genotype, CEM and their interactions with psychopathological status on clinical and behavioral data, SPSS 16.0 was used. Chi-square tests, *t*-tests and analysis of variance (ANOVA) were used where appropriate.

For the behavioral data, a repeated-measures ANOVA was conducted with RT as dependent variable, emotional expression as within-group factor and diagnosis, genotype and CEM as between-group factors.

fMRI data

Data were analyzed with SPM5, implemented in Matlab 7.1 (The MathWorks Inc.). Preprocessing included slice time correction, image realignment, registration of the T1 scan to the mean EPI, warping to MNI space as defined by the SPM5 T1-template, reslicing to 3 × 3 × 3 mm voxels and spatial smoothing using an 8-mm full with half maximum Gaussian kernel. Movement of the participant of >3 mm or rotation of >3° in any direction resulted in exclusion of this subject.

For every participant, hemodynamic responses for each stimulus were modeled. The model included regressors for each emotional expression (angry, fearful, happy, neutral and sad) and for baseline trials (scrambled faces). Low-frequency temporal noise was removed by applying a high pass filter of 128 s. For each participant, contrast images were calculated for ‘angry vs scrambled’, ‘fearful vs scrambled’, ‘sad vs scrambled’, ‘happy vs scrambled’ and ‘neutral vs scrambled’. We chose scrambled faces as our primary baseline condition, because a meta-analysis has shown that amygdala activation can be more reliably obtained by the use of a low-level baseline condition such as a

scrambled image compared with neutral facial stimuli as a baseline (Sergierie *et al.*, 2008). For completeness, we have also analyzed our data with the neutral condition as our baseline condition.

On a group level, we first performed a region of interest-based approach to test for the effects of genotype, CEM and diagnosis on amygdala activity. The individual contrast maps were entered in a group level analysis in a full-factorial model, with type of emotional facial expression added as within-subject factor. The individual signals of the entire bilateral amygdala were extracted from this full-factorial model using the MARSBAR toolbox (Brett *et al.*, 2002) for each contrast, and data were exported to SPSS for further analysis. The bilateral amygdala was defined according to the anatomical automatic labeling library (Maldjian *et al.*, 2003) implemented in the Wake Forest University pickatlas (<http://fmri.wfubmc.edu/cms/software>). A repeated-measures ANCOVA was performed on mean beta-values for all amygdala voxels with emotional expression and lateralization as within-subject variables and genotype, diagnosis and CEM as between-subject variables. Center was added as covariate of no interest. Main effects and interaction effects (*F*-tests) were regarded significant at $P < 0.05$. *Post hoc t*-tests were all Bonferroni corrected for multiple comparisons.

Second, an explorative whole-brain analysis was performed to test for additional brain regions where neuronal activity was related to NPY genotype *per se* or to an interaction between NPY genotype and CEM. Specifically, individual contrast maps were combined on a group level using ANOVA with emotional expression as within-subject factor and genotype and CEM as between-subject factors. MADRS scores and BAI scores were added as covariates to control for psychopathology. A threshold of $P < 0.005$ with an extent threshold of $k > 10$ for the *F*-tests was used to explore possible genotype effects. For *post hoc t*-tests, clusters were regarded significant at a threshold of $P < 0.05$ corrected for multiple comparisons using family-wise error (FWE) at cluster level.

For a description of psychiatric group differences in brain activity due to emotional facial processing in this sample, we refer to Demenescu *et al.* (2011) and for a description of main effects of CEM on brain activity, we refer to Van Harmelen *et al.* (2013).

RESULTS

Genotype data

In this sample, the genotype distribution over all subjects did not differ from the expected numbers calculated according to the HWE [$\chi^2(1) = 0.92$, $P > 0.25$].

There were relatively few T/T-homozygotes ([T/T:T/C:C/C] HC 3:16:14, MDD 4:17:12, ANX 6:10:5 and CAD 5:19:6). Therefore, to optimize power, we grouped the non-risk genotypes (T-allele carriers) and compared them with the risk genotype (C/C-genotype).

Clinical data

MADRS, BAI scores and psychiatric diagnosis were not related to genotype (all $P > 0.17$, Table 1). However, MADRS and BAI scores were both related to experienced CEM (all $P < 0.05$, Table 1). Both scores were higher in the CEM group than in the non-CEM group. There was also an effect of CEM on diagnosis: HC had experienced less CEM than patients (Table 1). There was an effect of genotype on experienced CEM. More non-risk-genotype carriers described CEM than risk-genotype carriers ($P = 0.002$).

Behavioral data

Due to technical problems, there was one participant for whom responses were not registered. Group mean substitution was used to analyze behavioral data of this participant. Three participants always

pressed the same button during baseline condition (responded to $> 89\%$ of items) and one participant did not respond to baseline items at all. For this last participant, the first-level contrasts on the fMRI data were thoroughly checked for abnormalities, which were not present. None of these participants were excluded. All other participants responded to $> 83\%$ of the stimuli and of the given responses $> 93\%$ were correct gender judgments.

There was a main effect of emotional expression on RT [$F_{(4, 98)} = 6.50$, $P < 0.001$]: participants responded faster to angry faces, than to the other expressions (data not shown). Over all emotional expressions, risk-genotype carriers responded faster than the non-risk-genotype carriers [$F_{(1, 101)} = 4.19$, $P = 0.04$, data not shown]. There were no main effects of diagnosis or CEM. However, there was an interaction between genotype and CEM [$F_{(1, 101)} = 5.17$, $P = 0.03$]: the fastest responses to the faces were given by those who carried the risk genotype and had experienced CEM (Figure 1). There was no interaction with diagnosis.

Imaging data

Effects on the amygdala

There was no main effect of genotype on amygdala activity [$F_{(1, 100)} = 0.08$, $P = 0.79$] nor an interaction between genotype and emotional expressions [$F_{(3.56, 356.2)} = 1.28$, $P = 0.28$]. However, an interaction between genotype and CEM was present on bilateral amygdala activity [$F_{(1, 100)} = 5.08$, $P = 0.026$]. Within risk-genotype carriers there was stronger amygdala activation for those who experienced CEM compared with those who did not, whereas there was no difference related to CEM within non-risk-genotype carriers (Figure 2). Emotional expressions of the stimuli did not influence this effect. There were no interactions between diagnosis and genotype, diagnosis and CEM nor a three-way interaction diagnosis, genotype and CEM.

There was a trend for an effect of diagnosis [$F_{(3, 100)} = 2.63$, $P = 0.05$], with ANX having less amygdala activation than HC and CAD. There was a main effect of CEM in the left and right amygdala across emotional expressions [$F_{(1, 100)} = 5.13$, $P = 0.026$]. Participants who experienced CEM had stronger amygdala activation compared with those who did not, irrespective of emotional expression. These findings are consistent with the findings of the larger sample reported elsewhere (Demenescu *et al.*, 2011; van Harmelen *et al.*, 2013).

To exclude the possibility that the interaction effect was driven by a concurrent history of physical and/or sexual abuse in some of the participants ($n = 24$), we next re-ran the RM ANCOVA while excluding these individuals. In this analysis, all results remained qualitatively unchanged, including the interaction effect of genotype and CEM [$F_{(1, 76)} = 5.35$, $P = 0.02$].

Except for the main effect of diagnosis, all the effects could be repeated in the larger sample in which patients using SSRIs were included (Supplementary Tables S1 and S2).

In addition, to explore the influence of our baseline condition, we repeated the analysis with the neutral face as baseline condition (instead of the scrambled face). In this analysis, only the main effect of CEM reached significance [$F_{(1, 101)} = 26.78$, $P = 0.02$].

There were no significant correlations between amygdala activity and RTs.

Exploratory whole-brain analysis into the effects of genotype and CEM

There were no additional significant effects related to genotype on regional brain activations in an analysis of HC and unmedicated patients. Explorative analyses showed a trend-wise interaction effect of genotype and CEM in the posterior cingulate cortex [PCC, ($x = 0$, $y = -45$, $z = 33$), $F_{(1, 450)} = 7.68$, $P = 0.006$]: those who experienced

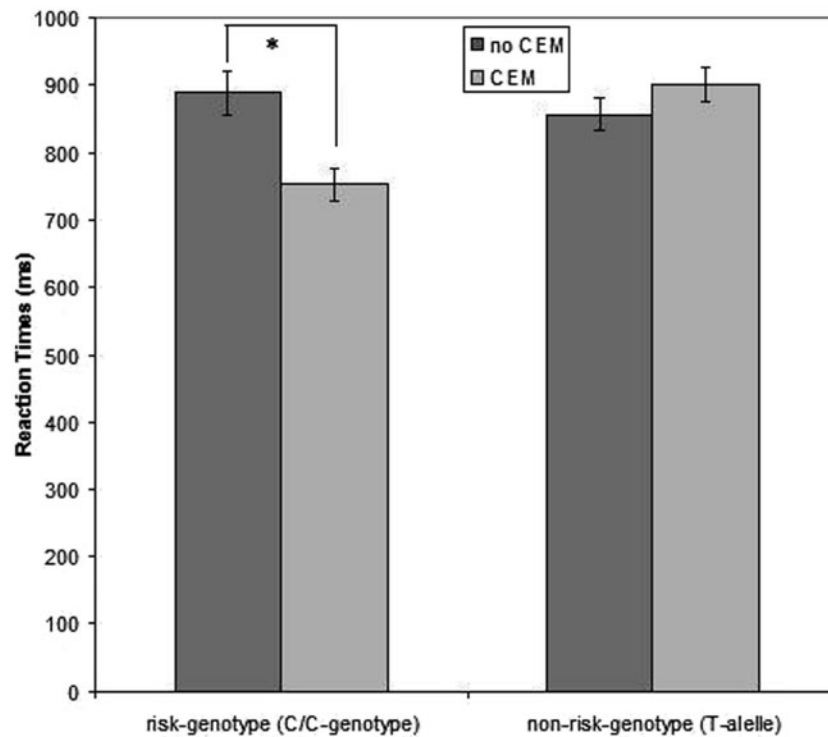


Fig. 1 RTs needed to judge the gender of the person in the photograph. This graph shows the combined effect of genotype and CEM on RTs. This effect was independent of the emotional facial expression depicted. The error bars represent one standard error.

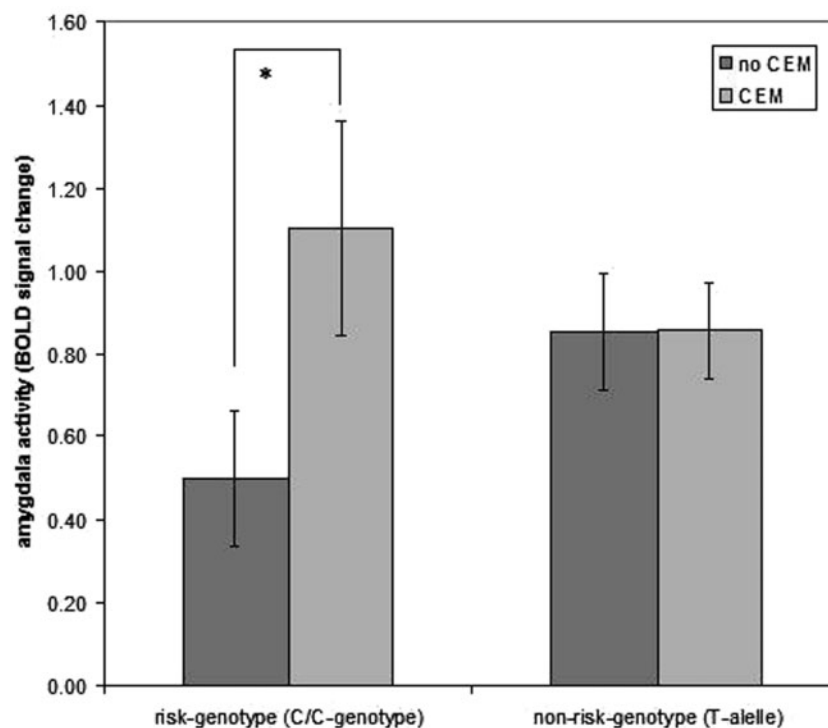


Fig. 2 Amygdala activity (for all faces relative to scrambled) in risk-genotype carriers (C/C-genotype) and non-risk-genotype carriers (T-allele) split according to the experience of CEM. Bars represent the mean (average over emotional expressions and left and right) and standard errors of amygdala activity. Within risk-genotype carriers there was increased amygdala activation for those who experienced CEM compared with those who did not [$t_{(36)} = 1.97$, $P = 0.02$], whereas there was no difference related to CEM with non-risk-genotype carriers [$t_{(78)} = 0.009$, $P = 0.99$]. The error bars represent one standard error.

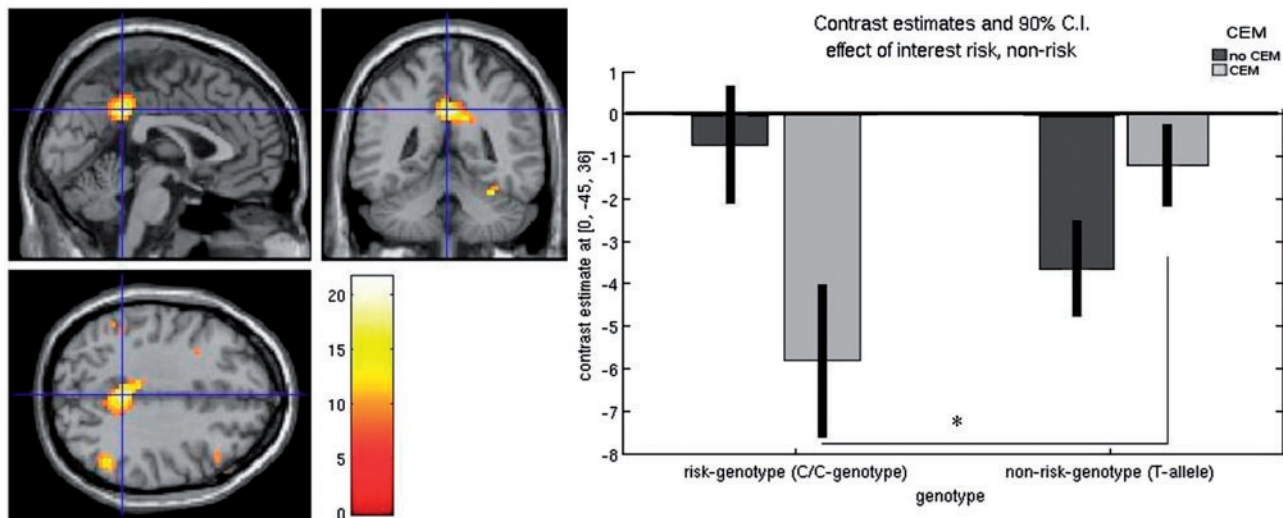


Fig. 3 PCC activation was also dependent on genotype and emotional maltreatment (like amygdala). Risk-genotype carriers who had experienced CEM had lower PCC activation compared with non-risk-genotype carriers ($Z = 3.82$, $P = 0.047$, FWE corrected on cluster level), whereas there was no such effect present in those who had not experienced emotional maltreatment. The difference between CEM and non-CEM within risk-genotype carriers did not reach significance ($Z = 3.68$, $P = 0.06$ uncorrected on cluster level). Figure shown at a threshold of $P = 0.001$.

CEM demonstrated lower activation of this region than individuals who did not reported a history of CEM. The number of subjects included in this study was likely not large enough to detect significant whole-brain effects. When also including the medicated patients (total sample size, $n = 165$), a significant interaction of genotype and CEM in the PCC, extending to the precuneus, was observed [$(x = 0, y = -45, z = 36)$, $F_{(1, 642)} = 21.59$, $Z = 4.46$, $k = 180$, $P < 0.001$ uncorrected, Figure 3]. Within participants who experienced CEM, carriers of the risk genotype had lower PCC activation compared with non-risk-genotype carriers ($Z = 3.82$, $P = 0.047$, FWE corrected on cluster level), whereas there was no such effect present in those who had not experienced CEM. The difference between CEM and non-CEM within risk-genotype carriers did not reach significance ($Z = 3.68$, $P < 0.001$ uncorrected and $P = 0.78$ FWE corrected). Including age and gender as covariates did not change the patterns of activation and association.

When comparing emotional faces with neutral faces instead of the scrambled faces control condition, there was no genotype by CEM interaction present.

DISCUSSION

The aims of this study were to investigate first the influence of NPY genotype on brain activity, second, whether this would interact with the experience of CEM and third, whether these effects are different in the presence of affective disorders. To examine whether such an interaction could contribute to vulnerability for affective disorders, we specifically investigated whether the combination of NPY risk genotype and CEM would impact on reactivity of brain areas involved in emotion processing. In addition, because of the possible associations between amygdala activity and affective psychopathology, we investigated whether the influence of NPY genotype and CEM on the brain is different in the presence of affective disorders.

Our results showed that CEM was associated with heightened amygdala reactivity within risk-genotype carriers, but not in carriers of the non-risk genotypes. This was accompanied by a faster motor response related to gender discrimination in those carrying the risk genotype and having experienced CEM, which was absent in non-risk-genotype carriers with CEM. The faster response together with the increased amygdala reactivity may point to hypervigilance for external emotional

stimuli. Because increased amygdala reactivity has been found in anxiety and depression (Matthews *et al.*, 2008; Peluso *et al.*, 2009; Townsend *et al.*, 2010; Victor *et al.*, 2010), our results might suggest a heightened vulnerability for these disorders carrying the risk allele and having a history of CEM.

Stronger amygdala activation in those carrying the risk genotype (C/C-genotype) and in addition having experienced CEM (Figure 3) is probably related to lower NPY levels. The C-allele of the rs16147 polymorphism has been associated with a reduction of NPY expression (Zhou *et al.*, 2008) and previous studies have also found increased amygdala activity within risk-genotype carriers (Zhou *et al.*, 2008; Domschke *et al.*, 2010). In addition, NPY levels are not only genetically determined but also depend on external factors. Animal models have shown that under conditions of stress, the expression and release of NPY is increased, implying successful behavioral adaptation (Thorsell *et al.*, 1999), but high levels of stress during childhood, such as maternal separation (Jimenez-Vasquez *et al.*, 2001; Husum *et al.*, 2002), have been shown to lead to reduced NPY levels. Recently, a decrease in NPY levels in the amygdala as a consequence of stress has also been demonstrated in humans (McGuire *et al.*, 2011). Thus, it could be hypothesized that NPY levels in those with risk genotype and experienced CEM are lowest and that these low NPY levels in the amygdala relate to increased amygdala activity.

The combination of having experienced stressful life events and carrying the NPY risk genotype has been found to increase vulnerability for affective disorders (Sommer *et al.*, 2010). The final aim of our study was to investigate whether presence of affective disorders moderated the effect of NPY. We did not find an interaction between diagnosis and NPY genotype or a three-way interaction including additionally CEM. Not finding an interaction with diagnosis could have been related to a relatively low statistical power to test for three-way interaction effects (e.g. relatively few healthy people experienced CEM and relatively few people with anxiety carried the risk genotype). Although we did not find an interaction with the presence of psychopathology, this gene by environment interaction on amygdala activation could be related to the onset of affective disorders through a disturbed stress response. Furthermore, the finding that the NPY genotype by CEM interaction was also observed in those who already experience affective psychopathology, may serve as part of a mechanism

by which patients are vulnerable for relapse, and thus ongoing course of the disorder. That is, amygdala reactivity could qualify as an endophenotype for the association between genetic and environmental influences and vulnerability for affective episodes.

An unexpected association was found between genotype and experienced CEM: carriers of the non-risk genotype reported CEM more often than risk-genotype carriers. It could be speculated that genotype is related to the subjective experience of CEM or the reporting of it. Notably, CEM was only measured by reports from the participants. Therefore, it could not be objectively verified if and to what degree CEM had occurred.

The direction of the effect of diagnosis on amygdala activity was unexpected with less activation in the amygdala for anxiety patients compared with both HC and comorbid patients, while previous studies mostly reported increased amygdala activation in anxiety patients [for a review, see reference Holzsneider and Mulert (2011)]. This could be related to chronically elevated amygdala activation or a strong reaction to scrambled faces, thus presenting a ceiling effect (Wright *et al.*, 2006).

SSRI use did not seem to influence the effects of genotype and CEM on neural activity. By including medicated patients the effect of diagnosis on amygdala activity disappeared. It could be suggested that medication attenuates amygdala activity, which is a commonly replicated finding in studies investigating the effects of SSRIs on brain activity (Sheline *et al.*, 2001; Fu *et al.*, 2004; Anand *et al.*, 2007; Ruhé *et al.*, 2012).

In the explorative whole-brain analysis, we observed a combined effect of NPY genotype and CEM on activity in the PCC extending to the precuneus. This effect was only seen in a larger sample including both SSRI-using and unmedicated patients. This finding may have been related to the effects of SSRI use on PCC activation, as there is one report of decreased PCC activation during self-referential processing after use of an SSRI [escitalopram (Matthews *et al.*, 2010)]. On the other hand, this finding may also have been due to increased power, because a similar trend was visible in the unmedicated sample only. This area showed stronger hypoactivity in response to human faces in participants carrying the risk genotype and having experienced CEM (compared with non-risk carriers who had experienced CEM, whereas the effect in the amygdala was compared with risk carriers without experienced CEM). The PCC and precuneus are part of the cortical midline structures and have been implicated in autobiographical memory (Cavanna and Trimble, 2006; Buckner and Carroll, 2007) and self-referential processes both emotionally and spatially (Dimaggio *et al.*, 2009; van der Meer *et al.*, 2010). Hypoactivity during processing of emotional faces can be interpreted as enhanced reactivity to external processes and less to self-related processing. In addition, this area has close connections with the amygdala (Veer *et al.*, 2011; Zhang and Li, 2012). Thus, our results suggest not only a gene by environment interaction in the amygdala but also for other regions connected in a broader neural network related to self-relevance and attention.

Interestingly, there were no differences related to genotype and/or CEM when neutral faces were used as baseline condition. Moreover, amygdala activation in response to neutral faces contrasted to the scrambled faces resulted in the same pattern of results as the emotional expressions contrasted against the scrambled face. These findings indicate that neutral faces elicit a comparable amygdala response as other emotions. This is in line with previous studies reporting amygdala reactivity to neutral stimuli (Wright and Liu, 2006; Blasi *et al.*, 2009). Moreover, also the 5-HTTLPR genotypes (coding for the serotonin transporter) have been shown to influence amygdala reactivity independent of the emotional expression, including neutral faces (Walsh *et al.*, 2012). It has been suggested that the ambiguity of neutral

stimuli (neither positive nor neutral) is potentially threatening and may elicit activation of the amygdala (Blasi *et al.*, 2009) and hypervigilance. Especially, patients with post-traumatic stress disorder (PTSD) have been shown to have strong amygdala responses to neutral faces (Brunetti *et al.*, 2010; Garrett *et al.*, 2012). Although PTSD was an exclusion criterion, subclinical PTSD might be present after CEM.

In addition, the effects were unrelated to type of emotional expression. This is contrary to the traditional idea of heightened amygdala activation for negative emotional expressions and the different social meaning of angry, fearful, sad, happy and neutral expressions. However, recent evidence suggests that the amygdala is not involved in processing of specific emotions, but has a general role in salience detection (Lindquist *et al.*, 2012) and the amygdala reacts similarly to positive and negative stimuli (Sergerie *et al.*, 2008). Our findings also suggest that in our sample various emotional expressions have a comparable salience for the subjects and elicit a similar response in the amygdala and PCC.

Although in a smaller sample, we replicated previous analyses carried out on data from the NESDA sample of an association between CEM and diagnosis (Hovens *et al.*, 2010; Spinhoven *et al.*, 2010) and of increased activity in the amygdala related to CEM (van Harmelen *et al.*, 2013). This suggests that the sample selection for this study reflects the larger NESDA sample. However, some limitations of this study should be mentioned. We could not replicate previously published findings regarding an association between NPY genotype and amygdala activity, regardless of CEM (Zhou *et al.*, 2008; Domschke *et al.*, 2010). In addition, a difference between our study and previous studies is the distribution of the genotypes in the sample. Especially, previous studies had more T/T-carriers in their sample, which could have made the difference in amygdala activity related to genotype larger than in our study. Our study was limited by a small HC-group with T/T-genotype and the lack of healthy T/T-carriers without CEM experience. Therefore, we decided to combine this group with the heterozygotes, to increase power, but this precluded the investigation of an additive effect of genotype. As mentioned earlier, assessment of CEM was based on self-report, so that it could not be objectively verified if and to what degree CEM had occurred. However, it has been shown that this method more likely leads to an underestimation than an overestimation of childhood abuse (Brewin, 2007). Moreover, a recent paper has shown that the risk for depression is associated with childhood maltreatment, but is independent of whether maltreatment was assessed prospectively or retrospectively (Scott *et al.*, 2012). A final limitation is that the cross-sectional design of this study precludes drawing causal inferences about CEM and amygdala response.

To conclude, in this study, we demonstrated an interaction of NPY genotype and CEM on amygdala and PCC activation during processing of emotional faces, in addition to a faster behavioral response. This is consistent with the notion that risk genotype plus CEM results in a hypervigilant state. This interaction could contribute to the vulnerability for developing affective disorders and confirms the relevance of gene-environment interactions on neurobiological mechanisms. Replication and further investigation are needed, to establish the role of NPY genotype in emotion processing networks in more detail.

SUPPLEMENTARY DATA

Supplementary data are available at SCAN online.

Conflict of Interest

E.M.O., R.K., M-J.T., S.W., M.A.B., B.W.Y.H.P. and D.J.V. declare no conflict of interest. N.J.A.W. received speaking fees from Eli Lilly and Wyeth; and served on advisory panels of Eli Lilly, Pfizer, Wyeth and Servier. A.A. received an investigator-initiated unrestricted research

grant from Bristol-Myers Squibb and speakers bureau honoraria from AstraZeneca, Bristol-Myers Squibb and GlaxoSmithKline. All these activities are not directly related to this study and, therefore, do not form a conflict of interest.

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