Amygdala responses to emotional faces in twins discordant or concordant for the risk for anxiety and depression.

Wolfensberger, S.P.A.; Veltman, D.J.; Hoogendijk, W.J.G.; Boomsma, D.I.; de Geus, E.J.C.

published in
NeuroImage
2008

DOI (link to publisher)
10.1016/j.neuroimage.2008.01.053

document version
Publisher's PDF, also known as Version of record

ELSEVIER

NeuroImage

www.elsevier.com/locate/ynimg
NeuroImage 41 (2008) 544–552

Amygdala responses to emotional faces in twins discordant or concordant for the risk for anxiety and depression

Saskia P.A. Wolfensberger,[a,c,*] Dick J. Veltman,[a,c] Witte J.G. Hoogendijk,[a,c] Dorret I. Boomsma,[b,c] and Eco J.C. de Geus[b,c]

[a]*Department of Psychiatry, Vrije Universiteit medical center, Amsterdam, The Netherlands*
[b]*Department of Biological Psychology, Vrije Universiteit, Amsterdam, The Netherlands*
[c]*Centre for Neurogenomics and Cognitive Research (CNCR), Amsterdam, The Netherlands*

Received 24 August 2007; revised 30 January 2008; accepted 31 January 2008
Available online 14 February 2008

Background: **Functional brain imaging studies have shown deviant amygdala responses to emotional stimuli in subjects suffering from anxiety and depressive disorder, but both hyperactivity and hypoactivity compared to healthy controls have been reported. To account for these discrepant findings, we hypothesize that genetic and environmental risk factors differently impact on amygdala functioning.**

Methods: **To test this hypothesis, we assessed amygdala responses to an emotional faces paradigm during functional magnetic resonance imaging in monozygotic twin pairs discordant for the risk of anxiety and depression ($n=10$ pairs) and in monozygotic twin pairs concordant for high ($n=7$ pairs) or low ($n=15$ pairs) risk for anxiety and depression.**

Results: **Main effects (all faces vs. baseline) revealed robust bilateral amygdala activity across groups. In discordant twins, increased amygdala responses were found for negatively valenced stimuli (angry/anxious faces) in high-risk twins compared to their low-risk co-twins. In contrast, concordant high-risk pairs revealed blunted amygdala reactivity to both positive and negative faces compared with concordant low-risk pairs. Post-hoc analyses showed that these findings were independent of 5-HTTLPR genotype.**

Conclusions: **Our findings indicate amygdala hyperactivity in subjects who are at high risk for anxiety and depression through environmental factors and amygdala hypoactivity in those at risk mainly through genetic factors. We suggest that this result reflects a difference in baseline amygdala activation in these groups. Future imaging studies on anxiety and depression should aim to avoid admixture of subjects who are at genetic risk with those at risk due to environmental factors.**

Keywords: fMRI; Amygdala; Faces; Twins; Anxiety; Depression

Introduction

Functional imaging studies of brain correlates of anxiety and depression have converged on the amygdala, a structure crucial for emotional processing (Canli et al., 2005; Davidson et al., 2003; Drevets, 2001; Fu et al., 2004; Kumari et al., 2003; Lawrence et al., 2004; Rauch et al., 2000; Shin et al., 2005; Siegle et al., 2001; Stein et al., 2002; Surguladze et al., 2005; Thomas et al., 2001; Wright et al., 2003). A replicated association has been found between amygdala reactivity to emotional stimuli and a polymorphism in the serotonin transporter gene (Hariri et al., 2002, 2005; Pezawas et al., 2005), and other genes in the serotonergic pathway also seem to influence amygdala reactivity (Brown et al., 2005; Buckholtz et al., 2007; Dannlowski et al., 2007; Iidaka et al., 2005). tioning. About 60% of the risk for anxiety and depressive disorders can be attributed to environmental factors (Kendler and Prescott, 1999; Sullivan et al., 2000) and interaction of genetic and environmental factors has also been reported (Caspi et al., 2000; Eley et al., 2004; Grabe et al., 2005; Kaufman et al., 2006; Kendler et al., 1986).

It is currently unclear whether environmental risk factors have the same pathogenic effects as genetic risk factors. It is quite possible that these two types of risk impact on the same brain structures but in entirely different perhaps even opposite ways. Indeed, deviant amygdala responses to emotional stimuli in subjects suffering from anxiety and depressive disorder have been observed, but although most studies have found hyperactivity compared to healthy controls (Canli et al., 2005; Fu et al., 2004; Siegle et al., 2001; Surguladze et al., 2005), in others negative findings (Kumari et al., 2003; Lawrence et al., 2004; Davidson et al., 2003), or even amygdala hypoactivity has been reported (Drevets, 2001; Thomas et al., 2001). Such discrepant findings may well arise because genetic and environmental risk factors differently impact amygdala functioning.

Here we employ brain imaging of concordant and discordant monozygotic (MZ) twin pairs to test how genetic and environmental

* Corresponding author. Department of Psychiatry, Nuclear Medicine and PET research, VU University Medical Centre, PO BOX 7057, 1007 MB Amsterdam, The Netherlands.
E-mail addresses: spa.wolfensberger@vumc.nl (S.P.A. Wolfensberger), dj.veltman@vumc.nl (D.J. Veltman).
Available online on ScienceDirect (www.sciencedirect.com).

1053-8119/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2008.01.053

risks for anxiety and depression are reflected in the amygdala response to emotionally salient stimuli. Monozygotic twins are (nearly) always 100% identical at the DNA sequence level (Boomsma et al., 2002) and differences in phenotypic status can be expected to reflect discordance for environmental risk factors. Discordant MZ twins were selected to be at low or high risk for anxiety disorder and major depression based on their ratings on neuroticism, anxiety, and depression in longitudinal surveys. A compound risk score for anxiety and depression based on these ratings was shown to have strong predictive validity for clinical anxiety and clinical depression in this population (Middeldorp et al., 2004) as assessed by the Composite International Diagnostic Interview, a well-validated instrument to assess these disorders (Andrews and Peters, 1998). Because MZ twins are genetically identical, discordance in the risk for anxiety and depression must arise from differential exposure to unique environmental influences. Our design allows us to test the hypothesis that these influences affect emotional processing by the amygdala.

In addition to discordant pairs, amygdala responses were assessed in two comparison groups of concordant monozygotic twin pairs. In the concordant high-risk group, both MZ twins scored high on neuroticism, anxiety, and depression in the longitudinal surveys. In the concordant low-risk group, both MZ twins had scored low on neuroticism, anxiety, and depression. The contrast between these two groups seems to mainly reflect a difference in genetic risk rather than in environmental risk because parents of the high-risk twins also scored very high on neuroticism, anxiety, and depression in the longitudinal surveys, whereas parents of the low-risk twins scored very low (De Geus et al., 2006). Comparison of the low and high scoring subjects allowed us to extend the finding of differential amygdala function in patients vs. controls to extreme scoring subjects in the normal population. Furthermore, comparing the contrast in amygdalar response between concordant high- and low-risk pairs to the within-pair contrast in discordant pairs allowed us to test whether genetic and environmental risk factors have a similar effect on amygdala responses to emotional stimuli.

Material and methods

Subjects

Selection of subjects has been described in detail by De Geus et al. (2006). Briefly, in a sample of 2455 same sex twin pairs registered in the Netherlands Twin Registry, a compound risk score for anxiety and depression was computed based on a genetic factor analysis of longitudinal survey data on trait anxiety, depression, neuroticism, and somatic anxiety (for details, see Boomsma et al., 2000). The surveys were collected in 1991, 1993, 1997, and 2000. An average risk score was computed for each twin across all available surveys (which could vary from one to four surveys).

Discordant MZ twins pairs were considered eligible for participation if both were right-handed, the high-risk subject had a risk score at least 0.5 standard deviation above the mean, and the score of high- and low-risk twins was at least 2 standard deviations apart. To estimate the relative risk for actual anxiety and depression disorder in twins ascertained by these criteria, we selected all subjects with comparable low- or high-risk scores from the larger sample of 1256 subjects that underwent a CIDI interview in 2000 (Middeldorp et al., 2004). To resemble the observed mean risk scores in our discordant twins (−0.62 vs. 0.97, SD=0.74), we selected subjects with a risk score of at least 1.5 standard deviation above the mean (1.06) on the 1991, 1993, and 1997 surveys and subjects with a risk score of at least 0.5 standard deviations below the mean (−0.32). In this sample, the relative risk to receive a lifetime depression diagnosis in the high scoring subjects was 11.8 compared with low scoring subjects and 40.1 for generalized anxiety disorder.

A total of 31 discordant MZ twin pairs met our criteria, of which we invited 17 pairs because they lived near Amsterdam and had filled out our most recent survey. Two pairs were excluded because one of the members had epilepsy or was pregnant. Four pairs refused to participate, mainly out of time constraints. One pair turned out to be dizygotic. This left a final 10 MZ pairs who were extremely discordant for the risk for anxiety and depression. Notably, the risk scores of the high-risk twin in these final 10 pairs did not differ from the risk scores in the high-risk twin from the original 31 selected pairs.

Concordant MZ twin pairs were considered eligible for participation if both were right-handed and both their risk scores were at least 0.8 standard deviation above (high risk) or below (low risk) the mean risk score. An even more stringent selection of extreme risk scores was possible here compared to the discordant MZ twin selection, because extreme scoring concordant MZ pairs are much less rare than extremely discordant pairs. This yielded 115 concordant high-risk and 137 concordant low-risk pairs, of which we invited 48 pairs that lived near Amsterdam and had filled out our most recent survey. Five pairs were excluded because one of the members had a medical illness or was pregnant. Twenty-one pairs refused to participate, mainly out of time constraints. This left a final 15 MZ pairs who were concordant for low risk and 7 MZ pairs who were concordant for high risk for anxiety and depression. Of the total group of 64 MZ, 28 were male and the mean age was 30 years (range 20–42 years).

Procedure

Subjects visited the outpatient MR unit and experimental procedures were explained in detail. Twins from the same pair always came on the same day. Twins were randomly assigned to an MRI scan session or a psychometric session. After about 90 min, twins switched between sessions. During the psychometric session, cognitive abilities and current psychiatric diagnostic state were assessed. All subjects were interviewed using the Composite International Diagnostic Interview (Peters and Andrews, 1995; Wittchen, 1994). The Montgomery Asberg Depression Rating Scale and the Beck Depression Inventory were used to assess depressive symptom characteristics and severity scores (Beck et al., 1961; Montgomery and Asberg, 1979). Furthermore, the state version of the State-Trait Anxiety Inventory (Spielberger et al., 1970) was administered pre- and post-scanning. Verbal comprehension (IQ) and working memory (forward and backward recall scores of digit scan) were assessed using subtests of the Wechsler Adult Intelligence Scale (Wechsler, 1997). Finally, social support was measured using the Duke–UNC questionnaire (Broadhead et al., 1988) and subjects were asked to recall the occurrence of 21 major life events. These included individual (e.g. maltreatment, disease, financial problems, job strain, relational problems) events as well as network-related events (e.g. disease or loss of close kin). Subjects were asked to locate these events in four temporal categories, i.e. whether they occurred the last six months, between 6 and 12 months, between 1 and 5 years, or more than 5 years ago. For each event, they indicated the impact on their lives on a 10-point visual analogue scale ranging from ‘no impact’ to ‘extreme impact’.

Table 1
Characteristics of the twins at the time of MRI scanning

	Low-risk concordant twins	Discordant twin pairs		High-risk concordant twins
		Low-risk twin	High-risk twin	
	N=30	*N*=10	*N*=10	*N*=14
Male/Female, no.	14/16	4/6	4/6	6/8
Age, mean, years	30.9	30.6	30.6	26.1
BDI depression, mean (SD)¶,*	1.1 (1.5)	2.7 (2.5)	9.7 (10.6)	8.0 (6.0)
MADRAS, mean (SD)¶,*	0.43 (1.5)	2.1 (2.5)	5.0 (3.9)	5.1 (7.8)
STAI State Anxiety before Scan session, mean (SD)¶,*	27.7 (5.0)	31.4 (5.3)	37.0(7.3)	36.7(9.8)
STAI State Anxiety after Scan session, mean (SD)¶,#,*	25.1 (4.7)	28.3 (5.5)	37.1 (8.1)	33.8 (8.4)
Verbal IQ subscale, mean (SD)	13.1 (2.5)	14.5 (2.2)	14.6 (2.2)	12.9 (3.9)
Working Memory IQ subscale, mean (SD)	7.6 (1.3)	8.9 (2.4)	8.3 (3.2)	8.6 (1.6)

¶ Significant difference between low-risk and high-risk concordants.
# Significant difference between high-risk subjects from concordant and discordant pairs.
* Significant intrapair difference in discordant twins.

During each MRI session, which lasted 45 min, subjects performed two tasks using emotionally relevant stimuli (faces and words). At first, a verbal memory task took place, followed by an emotional faces task. Between the encoding and recognition phase of the memory task, a structural MR scan was performed (De Geus et al., 2006). At the end of both sessions, subjects were debriefed and received sets of buccal swabs to collect mucosal cells for DNA extraction. DNA was used to confirm zygosity using 11 highly polymorphic markers and to type the *l/s* polymorphism in the promoter of the 5HT transporter gene. The 5-HTTLPR genotype was assessed in 26 MZ pairs. The ethical review board of the VU medical center approved the study and all participants provided written informed consent.

Task paradigm

We used an event-related emotional faces paradigm known to reliably activate the anterior medial temporal lobe including the amygdala (Wright et al., 2003). All subjects viewed human face stimuli: angry, fearful, happy, and neutral human faces (Ekman and Friesen, 2006), as well as a scrambled face, which was used as a baseline condition. Each face stimulus condition consisted of 10 pictures, and each picture was presented three times. Stimuli were randomized once and then presented in the same fixed order to all subjects. Stimuli were displayed for 2500 ms with a variable interstimulus interval (400–600 ms), to increase experimental power and to decrease expectancy effects. To control for overflow effects, we displayed a baseline stimulus after each one or two face pictures.

Subjects were requested to make sex judgments during presentation of face stimuli to control for attention differences. Baseline stimuli (scrambled faces) were imbedded with two arrows in the center of the screen, and subjects were asked to indicate whether the arrows pointed to the left or to the right. To ensure that participants were familiar with the procedure, the task was explained outside the scanner before fMRI was performed. No feedback regarding the answers was provided during the task.

Image acquisition

Magnetic resonance imaging was performed on a 1.5-T Sonata MR system (Siemens, Erlangen, Germany) with a standard RF receiver head coil. Stimuli were generated by a Pentium PC and projected on a screen at the end of the scanner table, which was seen through a mirror mounted above the subject's head. Two magnet-compatible four-key response boxes were used to record subject's performance and reaction times (RTs). To reduce motion artefacts, the subject's head was immobilized using foam pads.

For functional MRI, an echo planar imaging sequence (TR=3.04 s; TE=45 ms; matrix: 64×64; field of view: 192×192 mm; flip angle=90°) was used, creating transversal whole-brain acquisitions (35 slices, 3×3-mm in-plane resolution, 2.5-mm slice thickness, 0.5-mm interslice gap). In total, 214 EPI volumes per subjects were scanned. In addition, a coronal 3D gradient-echo T1- weighted sequence was performed (matrix: 256×256, inversion time: 300 ms, TR=15 ms, TE=7 ms; flip angle=8°; voxel size, 1×1×1.5 mm, 160 slices).

Data analysis

Differences in the questionnaire and interview based variables and in the performance data (reaction times) during the faces task were examined by a mixed model ANOVA (MIXED SPSS) with type of twin pair (Discordant, Concordant low-risk, Concordant high-risk) and risk score level (High, Low) as two fixed factors and

Table 2
Regions showing significantly increased brain activation across groups during fMRI scanning (faces vs. baseline)

Region (cortex)	MNI coordinates			*Z*-value	BA
	X	*Y*	*Z*		
DLPFC L	−48	15	27	3.59*	45
DLPFC R	51	12	27	4.57*	45
VLPFC L	−36	27	0	3.50*	46
VLPFC R	–	–	–	–	–
Anterior cingulate	−6	9	45	3.12*	24
Amygdala L	−21	−6	−18	4.86*	
Amygdala R	21	−6	−15	3.94*	
Thalamus R	−6	−9	−3	3.87*	
Temporo-occipital L	−39	−60	−18	6.55*	4
Temporo-occipital R	39	−57	−18	7.68*	4
Occipital	−33	−90	−12	5.51*	18/19
	36	−87	−9	5.92*	
	−6	−93	9	7.08*	17

* Significant FDR corrected at $p<0.05$.
Cluster size threshold, 5 voxels; BA, Brodmann area; DLPFC, dorsolateral prefrontal cortex; L, left; R, right; VLPFC, ventrolateral prefrontal cortex.

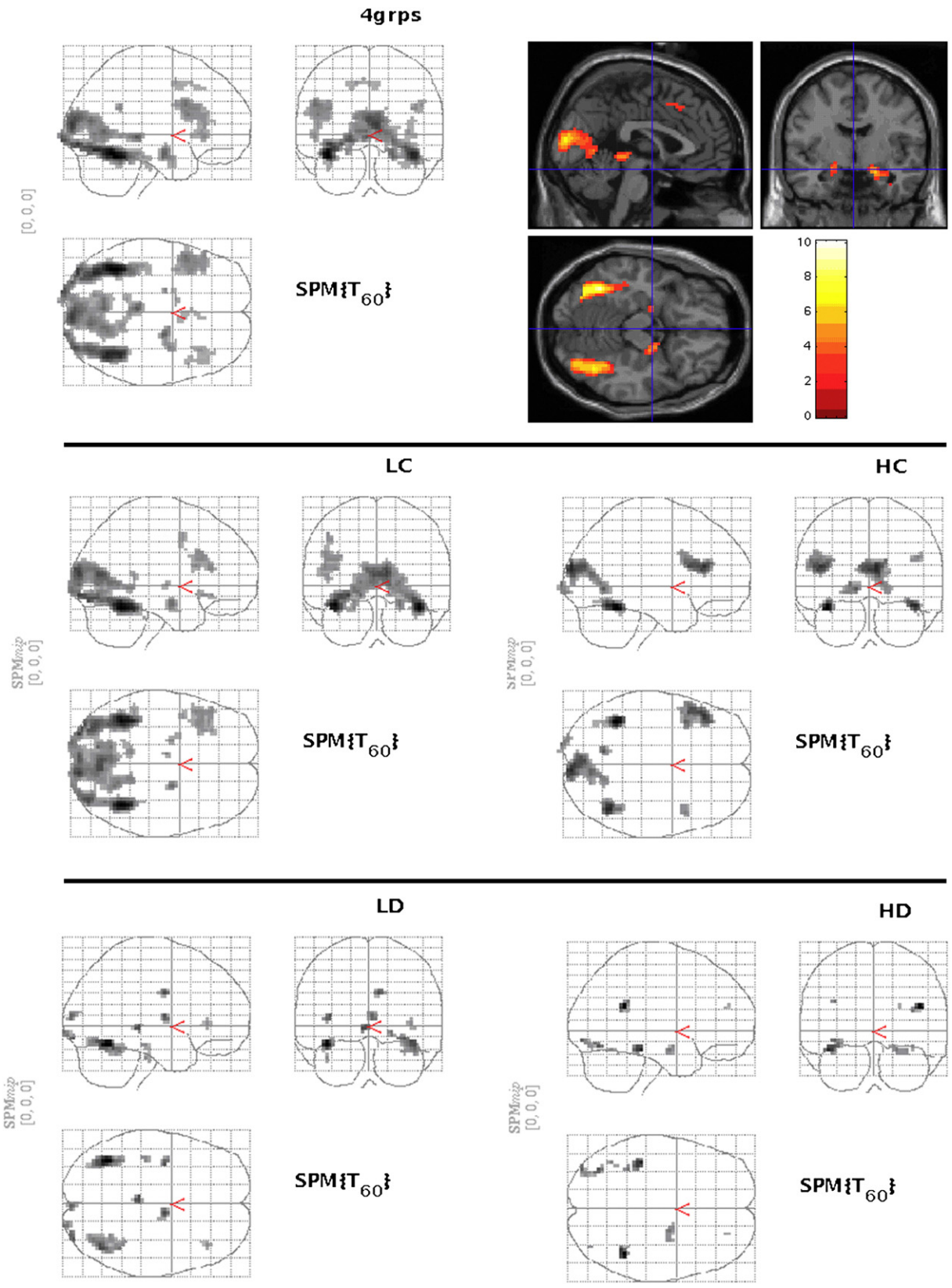

Fig. 1. Main effect of faces (all faces vs. baseline) across all 4 experimental groups (upper left panel: glass brain; upper right panel: overlaid on T1-weighted anatomical image) and for the subgroups of the concordant low-risk pairs (LC, middle left panel), the concordant high-risk pairs (HC, middle right panel), the discordant low-risk twins (LD, lower left panel), and their high-risk co-twins (HD, lower right panel).

Table 3
Comparison of the amygdala effects (faces vs. baseline) in low-risk and high-risk twins

Condordant pairs			
Concordant low-risk twins > concordant high-risk twins			
Contrast	Region	MNI coordinates	*Z*-score
All faces vs. baseline	L amygdala	−18 −3 −15	2.59#
	R amygdala	27 −6 −24	3.15*
		21 0 −6	2.97*
Angry/anxious faces vs. baseline	R amygdala	27 −3 −24	3.39*
Happy faces vs. baseline	R amygdala	24 0 −24	2.91*
Discordant pairs			
High risk twin > low risk co-twin			
Contrast	Region	MNI coordinates	*Z*-score
All faces vs. baseline	L amygdala	−21 0 −21	2.67*
Angry/anxious faces vs. baseline	L amygdala	−30 −6 −21	2.71*

* Results reported significant at uncorrected $p<0.005$, cluster size threshold 5 voxels.
# $p=0.0054$.

family as a random factor to account for within-family dependence. Primary planned contrasts were the comparison of the low-risk vs. the high-risk co-twin within the 10 discordant pairs, and of the 7 concordant high-risk vs. the 15 concordant low-risk pairs.

Imaging data were analyzed using SPM2 (Statistical Parametric Mapping; Wellcome Department of Cognitive Neurology, London, UK). After the first two volumes were discarded to allow for magnetic saturation, time series were corrected for differences in slice acquisition times and realigned. Next, data were warped to MNI space as defined by the SPM EPI template, and spatially smoothed using an 8 mm FWHM Gaussian kernel. After spatial preprocessing, data were analyzed using delta functions convolved with a canonical hemodynamic response function to model responses to each stimulus type. For each subject, weighted contrasts were computed for simple main effects. This was done across all stimulus types, i.e. viewing angry/anxious, happy, and neutral faces vs. baseline, and within stimulus type, e.g. angry/anxious vs. baseline. The resulting contrasts images were entered into second level (random effects) analyses for between-group comparisons. These used a one-way ANOVA for the groups of concordant high- vs. low-risk pairs (Group) and paired *t*-tests for the high-risk twin vs. the low-risk co-twin within discordant pairs (Twin). SPM2's non-sphericity option was used to account for within-pair correlated observations. Main effects of risk status (Group or Twin) are reported at $p<0.05$ FDR- corrected for multiple comparisons with an extent threshold of five voxels (Genovese et al., 2002). Interactive effects of Risk by stimulus type (angry/anxious vs. baseline, happy vs. baseline, neutral vs. baseline; masked with the relevant main effect) within the amygdala region are reported at $p<0.005$ uncorrected, also with an extent threshold of five voxels.

Results

Demographic data and depression/anxiety ratings (BDI, MADRS, STAI-state) obtained at the time of the scanning session are listed in Table 1. Age and male/female distribution was not significantly different across the groups. Only one subject in a concordant high-risk pair received a current diagnosis of depression using these instruments. One further subject currently used antidepressant medication (SSRI). This was the subject with the high-risk score from a discordant pair. Mixed ANOVA confirmed that the concordant low-risk pairs scored significantly lower on the MADRS, BDI, and state anxiety measures than the concordant high-risk pairs. Within the discordant pairs, the MADRS, BDI, and state anxiety measures all showed significant intra-pair differences in the expected direction.

Reaction times during the task (sex judgments) for angry, anxious, happy, neutral faces, and across categories did not differ significantly between groups (mean RT ± SD: angry faces, 800 ± 171 ms; anxious faces, 787 ± 170 ms; happy faces, 794 ± 172 ms; neutral faces, 769 ± 173 ms; across categories, 787 ± 166 ms; baseline, 698 ± 132 ms).

Imaging data

Effects of faces

Across groups, viewing faces compared to baseline were associated with robust activity in bilateral occipital cortex, extending into fusiform gyrus and posterior parahippocampal gyrus, as well as in

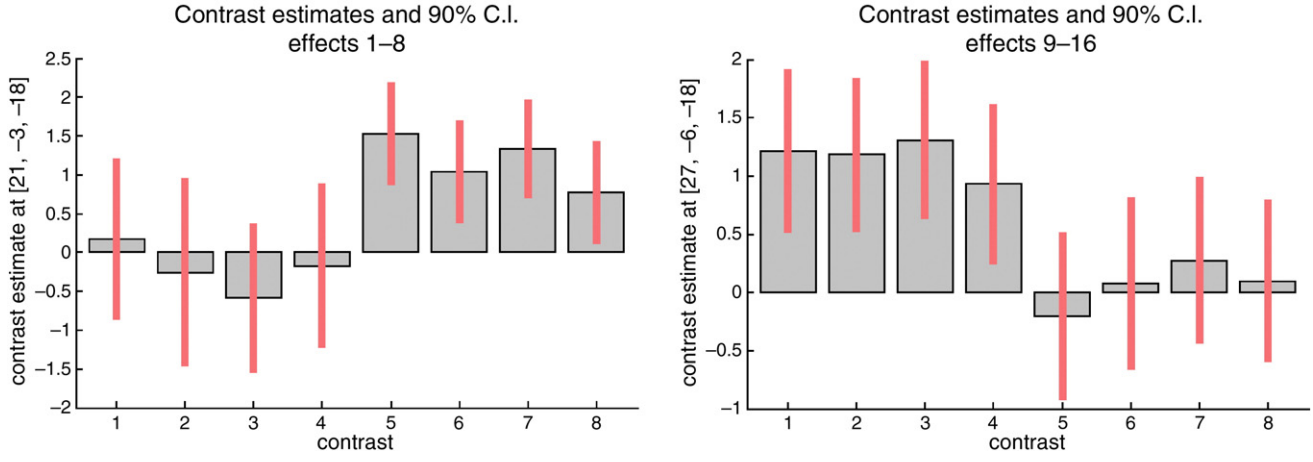

Fig. 2. Left panel: parameter estimates (% signal change) and 90% confidence intervals for angry/anxious/happy/neutral faces vs. baseline (scrambled faces) in concordant high-risk (effects 1–4) vs. concordant low-risk twins (effects 5–8). Right panel: parameter estimates (% signal change) and 90% confidence intervals for angry/anxious/happy/neutral faces vs. baseline (scrambled faces) in concordant high-risk (effects 9–12) vs. concordant low-risk twins (effects 13–16).

bilateral amygdala, with additional activity in predominantly left lateral prefrontal cortex, dorsal anterior cingulate cortex, and thalamus (Table 2). These effects are depicted across groups (Fig. 1, upper panel) and for individual groups (Fig. 1, middle and lower panels).

Within-pair comparison of the discordant twins

Across the neutral, happy, and angry/anxious faces, paired t-tests comparing the low-risk twin to the high-risk co-twin showed increased left amygdala activity in the high-risk twin (Table 3). Risk × stimulus type interaction analyses in discordant twins revealed increased left amygdala activity for angry/anxious faces in high-risk twin compared to the low-risk co-twin, whereas this comparison failed to reach significance for happy or neutral faces (Fig. 2, left panel). High-risk twins had substantially higher state anxiety levels before the scan compared to their low-risk co-twin (see Table 1). Rerunning the analyses using these state anxiety levels as a covariate rendered all amygdala effects non-significant.

Group comparison of concordant high-risk and low-risk twin pairs

Across the neutral, happy, and angry/anxious faces, we observed increased activity over baseline in bilateral amygdala for concordant low-risk pairs compared to high-risk pairs (using one-way ANOVA), with left amygdala approaching significance (Table 3). Risk × stimulus type interaction analyses showed increased amygdala activity in low-risk pairs compared to the high-risk pairs for angry/anxious faces and happy faces relative to baseline, but not for neutral faces (Fig. 2, right panel). In contrast to the findings in discordant twins, rerunning the analyses using state anxiety levels before the scan as a covariate left all group differences in amygdala reactivity significant.

Additional post hoc analyses showed highly similar results after excluding the subject meeting criteria for clinical depression and the subject using antidepressants. In addition, in a secondary analysis, we failed to observe amygdala activity when contrasting baseline trials with neutral faces in either the comparison of the low-risk twin with the high-risk co-twin in discordant pairs or in the comparison of concordant low-risk and high-risk pairs.

5-HTTLPR genotypes

Previous studies have reported differential amygdala responsivity in subjects with different genotypes for a functional polymorphism in the gene coding for the serotonin transporter (LL vs. LS vs. SS; Munafò et al., 2007). Because the frequency of the LL genotype in the concordant high-risk pairs is high compared to that found in unselected samples, part of the attenuated amygdale response may have reflected effects of this particular polymorphism. To test whether our findings reflected effects of the 5-HTTLPR genotype, we first examined the 5-HTTLPR genotype distribution across groups (Table 4). Permutation tests showed that 5-HTTLPR genotype was not related to twin depression status (low or high risk for anxiety and depression). Next, we examined whether the amygdala response differed as a function of genotype by contrasting the BOLD responses of the LL ($N=8$ pairs) and SL/SS ($N=18$ pairs) 5-HTTLPR genotypes. This was done independent of risk or concordance status, i.e. pooled across the four groups. No significant differences between genotype groups were found.

Table 4
5-HTTLPR genotype in the three types of MZ twin pairs

	5-HTTLPR genotype			
	LL	LS/SL	SS	Total
Discordant twin pairs	4	12	2	18
Concordant high-risk pairs	4	4	0	8
Concordant low-risk pairs	8	14	4	26
Total pairs	16	30	6	52

Discussion

The main aim of this study was to investigate differences in amygdala response to emotional faces in relation to a "pure" environmental risk for depression and anxiety and to contrast this with the amygdala response in relation to genetic risk. All subjects viewed positively and negatively valenced emotional and neutral faces during functional MR imaging. Analysis of imaging data revealed robust activation in visual processing areas including the fusiform face area as well as in bilateral amygdala, left ventrolateral prefrontal cortex, and bilateral dorsolateral prefrontal cortex, the latter presumably reflecting sex categorization (Adams and Janata, 2002). These results are in agreement with a number of previous studies using a similar paradigm. Specifically, although in some studies predominantly right-sided responses were observed, irrespective of emotion (Gur et al., 2002), in others bilateral amygdala responses were found across stimulus conditions (Britton et al., 2006; Fitzgerald et al., 2006; Yang et al., 2002), similar to the present study.

In discordant MZ twin pairs, increased left amygdala responsiveness was found in the high-risk twin for angry/anxious faces. As MZ twins are nearly always genetically identical, this amygdala hyperactivity in the high-risk twin necessarily reflects the role of environmental factors. Indeed, as reported previously (De Geus et al., 2006), nine out of the ten high-risk twins from the discordant pairs reported major life events that occurred more than 5 years ago, and 5 twins reported multiple events. From the low-risk co-twins, only four reported such events, and only one reported multiple events. In addition, the average impact of the life events was generally rated to be larger by the high-risk twins. In keeping with these findings, amygdala hyperactivity in response to faces has been found in posttraumatic stress disorder (Rauch et al., 2000; Siegle et al., 2001), in which life events are by definition of aetiological significance, but also in social phobia and generalized anxiety disorder (Stein et al., 2002), in which the role of genetic factors may be only modest (Middeldorp et al., 2004; Mackintosh et al., 2006). In contrast, normal or even lowered amygdala responses to emotional faces have been reported in simple phobias (Wright et al., 2003) and obsessive–compulsive disorder (Cannistraro et al., 2004).

At odds with our findings in discordant twins, the concordant high-risk twin pairs had a blunted amygdala response to emotional faces. Assessment of parental data for all pairs confirmed higher values for all risk traits in the parents of the concordant high-risk twins compared to the parents of the concordant low-risk twins (De Geus et al., 2006). Although additional exposure of the high-risk twin pairs cannot be excluded, this strongly suggests that the concordant pairs mainly represent a contrast in genetic risk. Hence, we observed a clear dissociation in the amygdala responses of subjects who were at high-risk for anxiety and depression through environmental causes (hyperactivity) compared to those mostly at risk through genetic factors (hypoactivity).

These clear differences in the effects of genetic and environmental risk factors might help explain the inconsistency in the outcome of previous fMRI studies using similar emotional paradigms in MDD patients and controls. Increased responses to negative emotional stimuli in depressed subjects were observed by several investigators (Canli et al., 2005; Fu et al., 2004; Siegle et al., 2001; Surguladze et al., 2005), although decreased responding to positive emotional stimuli (happy faces) has also been found, which was associated with anhedonia severity (Surguladze et al., 2005). Other groups, however, failed to observe differential amygdala activity (Davidson et al., 2003; Kumari et al., 2003; Lawrence et al., 2004) or even reported a blunted response, in depressed children (Thomas et al., 2001) and familial depression (Drevets, 2001), respectively. These inconsistencies have been attributed to methodological issues (e.g. medication use), or differences in state anxiety (Sheline et al., 2001). In the present study, medication use was virtually absent in the high-risk twins which rules this out as an explanation here. Anxiety ratings were high in both discordant high-risk twins and concordant high-risk twin pairs which suggest that state anxiety cannot fully account for the contrast between hyporeactivity and hyperreactivity. Instead, genetic and environmental risk factors, although converging on the same pathway, seem to exert different effects on amygdala functioning. Hence, differential exposure of patients to genetic or environmental risk factors, perhaps related to ascertainment methods, may account for part of the differential findings in the literature.

Previous research in genetically contrasted groups has revealed larger amygdala activity in response to emotional faces in subjects with one or two copies of the short allele of the serotonin transporter promoter (HTTLPR) polymorphism (Bertolino et al., 2005; Hariri et al., 2002, 2005). Since the short allele carriers have an increased risk for anxiety and depression compared to long allele homozygotes, particularly in interaction with life stress (Caspi et al., 2003), it might be argued that our findings could be explained by differential genotypes for the serotonin transporter gene in the three groups of twins (concordant low-risk, concordant high-risk, discordant). This was not the case, as there was no difference in the distribution of the HTTLPR alleles between the three groups. This is not without precedent since amygdala responsiveness has previously been found to be modulated by personality style (Bertolino et al., 2005) and harm avoidance (Hariri et al., 2005), independent of the 5-HTTLPR genotype. Also, a direct test of 5-HTTLPR genotype effects on the amygdala response was non-significant. This may reflect the small sample size, but null findings have been noted before (Munafò et al., 2007).

This leaves us to explain the attenuated amygdala responses in the concordant high-risk twins, a group selected to be at high genetic risk for anxiety and depression. Based on the proposed inverse relationship between amygdala reactivity and baseline amygdala activation (Canli et al., 2006), we hypothesize that this genetic risk co-occurs with an increase in baseline amygdala activation. This is in keeping not only with recent perfusion MR-findings (Canli et al., 2006) showing increased baseline amygdala perfusion in subjects with decreased responsiveness to emotional stimuli, but also with SPECT- and PET-resting-state studies in familial MDD patients by Drevets and coworkers (Drevets, 2000a,b, 2001, 2003). A consistent finding in these latter studies has been elevated baseline amygdala perfusion and/or glucose uptake in patients with familial MDD (Drevets, 2000a,b, for an overview). The mechanism of increased amygdala baseline metabolism in familial MDD is insufficiently understood, but may reflect both altered afferent transmission (increased glutamatergic input from ventral prefrontal areas and/or decreased inhibitory control from dorsal medial PFC) as well as a shift from ISPS to ESPS within the amygdala due to elevated CRF secretion (Drevets, 2003; Phillips et al., 2003; Shekhar et al., 2003). Animal research has indicated that the increase in regional CBF or metabolism may be as high as 50–70% (Drevets, 2003). Although this is still in the physiological range, it is conceivable that a rise in baseline perfusion of such magnitude may compromise amygdala responsiveness during processing of emotional stimuli. At present, such an explanation is speculative, since studies in MDD assessing both amygdala resting state perfusion and responsiveness to emotional stimuli have been rare (Anand et al., 2005). Moreover, it should be taken into account that only one of our concordant high-risk twins met criteria for major depression at the time of scanning, so that extension of our findings of blunted amygdala responsiveness to clinical studies should be done with caution.

Various limitations of this study should be discussed. First, it is important to note that, although our selection of high- and low-risk twins was based on trait measures, the high- and low-risk twins were also strongly discriminated in state anxiety levels at the time of testing. This is an expected outcome. Individuals high on trait scores for neuroticism, anxiety, and depression are expected to respond to the study setting (unfamiliar environment, imperfect prediction of things to come, unknown experimenter, intimidating MRI apparatus, etc.) with more anxiety then individuals with low scores for these traits. It is of note that these state anxiety differences do not escape the basic tenet of our design: they will derive from differential environmental exposure in the discordant pairs and mainly derive from a difference in genetic make-up in the concordant pairs. Nonetheless, the effects of risk status defined by the longitudinal assessed trait values were obviously confounded with the effects of state anxiety. In an attempt to separate the effects of trait from state effects, we reran the analyses using state anxiety before the scan as a covariate. This was expected to largely undo the effects of our careful longitudinal selection for high trait values in both genetic and environmental contrasts. Instead, hypoactivity in the high-risk concordant twins remained significant, whereas hyperactivity of the high-risk discordant twin disappeared. This suggests that high state anxiety is responsible for amygdala hyperactivity in subjects at risk through environmental factors. They behave as healthy subjects deliberately made anxious by employing aversive conditioning paradigms. Increased amygdala responsiveness has consistently been observed in such paradigms (Buchel et al., 1998, 1999; Schienle et al., 2005). In contrast, the increased baseline activation in subjects at genetic risk seems to completely overrule these normal effects of state anxiety. Only a design that manages to assess the effects of state and trait anxiety independently can fully resolve this issue.

As a second limitation, it should be noted that blunted amygdala responses in high-concordant subjects could be due to increased amygdala responsiveness to the baseline trials, as was recently demonstrated by Heinz et al. (2007). In their study, these authors showed that in s-allele carriers, amygdala activation was increased during passively viewing a fixation cross relative to neutral stimuli, explained as resulting from experiencing (stressful) uncertainty. However, in the present study, post-hoc analyses failed to show amygdala activity when contrasting baseline trials with neutral faces. We avoided the use of unconstrained baseline trials (Stark and Squire, 2001) by requesting subjects to report the direction of arrows (overlaid on scrambled faces) which apparently attenuates the effect of the uncertainty generated by fixation cross type baseline stimuli.

Finally, we acknowledge that the discordant MZ twins may not have been perfect genocopies. A number of rare occurrences can lead

to genetic differences between MZ twins (Martin et al., 1997) and such MZ twin pairs may be enriched in a sample that is highly selected for trait discordance. If these MZ twins were not genetically identical this would question the role of the environment in making them discordant. In contrast to this idea and in keeping with true differences in environmental exposure, we found that the high-risk discordant twins had been exposed to adverse life events more often than their low-risk co-twin. Also, the most likely source of genetic differences within twin-pairs, epigenetics, seems to be primarily driven by differential environmental exposure itself (Fraga et al., 2005).

In summary, we observed decreased amygdala reactivity to emotional (positive and negative) faces in twins at high genetic risk for anxiety and depression, whereas increased responsiveness to negatively valenced faces was observed in twins at high environmental risk. We suggest that this result reflects a difference in baseline amygdala activation in these two groups. Further research should aim to elucidate pathophysiological mechanisms of amygdala dysfunction in genetically vs. environmentally driven anxiety and depression. In addition, future imaging studies on anxiety and depression should avoid admixture of subjects who are at risk due to genetic factors with those at risk due to environmental factors.

Acknowledgments

This study was supported by the Netherlands Organization for Scientific Research (NWO) grants 900-562-137, 904-61-090, 985-10-002, 904-61-193, 480-04-004, and 575-25-006, the Centre for Neurogenomics and Cognitive Research (CNCR), and the Centre for Medical Systems Biology (CMSB), a center of excellence approved by the Netherlands Genomics Initiative/NWO. We acknowledge the valuable contribution of Marcel Jansen and Kim Baas to data collection in the twins.

All authors had no conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject of their manuscript.

References

Adams, R.B., Janata, P., 2002. A comparison of neural circuits underlying auditory and visual object categorization. NeuroImage 16, 361–377.

Anand, A., Li, Y., Wang, Y., Wu, J.W., Gao, S.J., Bukhari, L., Mathews, V.P., Kalnin, A., Lowe, M.J., 2005. Activity and connectivity of brain mood regulating circuit in depression: a functional magnetic resonance study. Biol. Psychiatry 57, 1079–1088.

Andrews, G., Peters, L., 1998. The psychometric properties of the Composite International Diagnostic Interview. Soc. Psychiatry Psychiatr. Epidemiol. 33, 80–88.

Beck, A.T., Erbaugh, J., Ward, C.H., Mock, J., Mendelsohn, M., 1961. An inventory for measuring depression. Arch. Gen. Psychiatry 4, 561–571.

Bertolino, A., Arciero, G., Rubino, V., Latorre, V., De Candia, M., Mazzola, V., Blasi, G., Caforio, G., Hariri, A., Kolachana, B., Nardini, M., Weinberger, D.R., Scarabino, T., 2005. Variation of human amygdala response during threatening stimuli as a function of 5'HTTLPR genotype and personality style. Biol. Psychiatry 57, 1517–1525.

Boomsma, D.I., Beem, A.L., van den, B.M., Dolan, C.V., Koopmans, J.R., Vink, J.M., de Geus, E.J., Slagboom, P.E., 2000. Netherlands twin family study of anxious depression NETSAD. Twin Res. 3, 323–334.

Boomsma, D., Busjahn, A., Peltonen, L., 2002. Classical twin studies and beyond. Nat. Rev., Genet. 3, 872–882.

Britton, J.C., Taylor, S.F., Sudheimer, K.D., Liberzon, I., 2006. Facial expressions and complex IAPS pictures: common and differential networks. NeuroImage 31, 906–919.

Broadhead, W.E., Gehlbach, S.H., Degruy, F.V., Kaplan, B.H., 1988. The Duke-Unc Functional Social Support Questionnaire—measurement of social support in family medicine patients. Med. Care 26, 709–723.

Brown, S.M., Peet, E., Manuck, S.B., Williamson, D.E., Dahl, R.E., Ferrell, R.E., Hariri, A.R., 2005. A regulatory variant of the human tryptophan hydroxylase-2 gene biases amygdala reactivity. Mol. Psychiatry 10, 884–888.

Buchel, C., Dolan, R.J., Armony, J.L., Friston, K.J., 1999. Amygdala-hippocampal involvement in human aversive trace conditioning revealed through event-related functional magnetic resonance imaging. J. Neurosci. 19, 10869–10876.

Buchel, C., Morris, J., Dolan, R.J., Friston, K.J., 1998. Brain systems mediating aversive conditioning: an event-related fMRI study. Neuron 20, 947–957.

Buckholtz, J.W., Callicott, J.H., Kolachana, B., Hariri, A.R., Goldberg, T.E., Genderson, M., Egan, M.F., Mattay, V.S., Weinberger, D.R., Meyer-Lindenberg, A., 2007. Genetic variation in MAOA modulates ventromedial prefrontal circuitry mediating individual differences in human personality. Mol. Psychiatry 10, 893–895.

Canli, T., Cooney, R.E., Goldin, P., Shah, M., Sivers, H., Thomason, M.E., Whitfield-Gabrieli, S., Gabrieli, J.D.E., Gotlib, I.H., 2005. Amygdala reactivity to emotional faces predicts improvement in major depression. NeuroReport 16, 1267–1270.

Canli, T., Qiu, M., Omura, K., Congdon, E., Haas, B.W., Amin, Z., Herrmann, M.J., Constable, R.T., Lesch, K.P., 2006. Neural correlates of epigenesis. Proc. Natl. Acad. Sci. U. S. A. 103, 16033–16038.

Cannistraro, P.A., Wright, C.I., Wedig, M.M., Martis, B., Shin, L.M., Wilhelm, S., Rauch, S.L., 2004. Amygdala responses to human faces in obsessive–compulsive disorder. Biol. Psychiatry 56, 916–920.

Caspi, A., Taylor, A., Moffitt, T.E., Plomin, R., 2000. Neighborhood deprivation affects childrens mental health: Environmental risks identified in a genetic design. Psychol. Sci. 11, 338–342.

Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A., Poulton, R., 2003. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301, 386–389.

Dannlowski, U., Ohrmann, P., Bauer, J., Deckert, J., Hohoff, C., Kugel, H., Arolt, V., Heindel, W., Kersting, A., Baune, B.T., Suslow, T., 2007. 5-HTTLPR biases amygdala activity in response to masked facial expressions in major depression. Neuropsychopharmacology 33, 418–424.

Davidson, R.J., Irwin, W., Anderle, M.J., Kalin, N.H., 2003. The neural substrates of affective processing in depressed patients treated with venlafaxine. Am. J. Psychiatry 160, 64–75.

De Geus, E.J., Ent, D.V., Wolfensberger, S.P., Heutink, P., Hoogendijk, W.J., Boomsma, D.I., Veltman, D.J., 2006. Intrapair differences in hippocampal volume in monozygotic twins discordant for the risk for anxiety and depression. Biol. Psychiatry 61, 1062–1071.

Drevets, W.C., 2000a. Functional anatomical abnormalities in limbic and prefrontal cortical structures in major depression. Prog. Brain Res. 126, 413–431.

Drevets, W.C., 2000b. Neuroimaging studies of mood disorders. Biol. Psychiatry 48, 813–829.

Drevets, W.C., 2001. Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. Curr. Opin. Neurobiol. 11, 240–249.

Drevets, W.C., 2003. Neuroimaging abnormalities in the amygdala in mood disorders. Ann. N.Y Acad. Sci. 985, 420–444.

Ekman, P., Friesen, W., 2006. Pictures of Facial Affect. Consulting Psychologists Press, Palo Alto.

Eley, T.C., Sugden, K., Corsico, A., Gregory, A.M., Sham, P., McGuffin, P., Plomin, R., Craig, I.W., 2004. Gene-environment interaction analysis of serotonin system markers with adolescent depression. Mol. Psychiatry 9, 908–915.

Fitzgerald, D.A., Angstadt, M., Jelsone, L.M., Nathan, P.J., Phan, K.L., 2006. Beyond threat: amygdala reactivity across multiple expressions of facial affect. NeuroImage 30, 1441–1448.

Fraga, M.F., Ballestar, E., Paz, M.F., Ropero, S., Setien, F., Ballestart, M.L., Heine-Suner, D., Cigudosa, J.C., Urioste, M., Benitez, J., Boix-Chornet, M., Sanchez-Aguilera, A., Ling, C., Carlsson, E., Poulsen, P., Vaag, A., Stephan, Z., Spector, T.D., Wu, Y.Z., Plass, C., Esteller, M., 2005. Epigenetic differences arise during the lifetime of monozygotic twins. Proc. Natl. Acad. Sci. U. S. A. 102, 10604–10609.

Fu, C.H.Y., Williams, S.C.R., Cleare, A.J., Brammer, M.J., Walsh, N.D., Kim, J., Andrew, C.M., Pich, E.M., Williams, P.M., Reed, L.J., Mitterschiffthaler, M.T., Suckling, J., Bullmore, E.T., 2004. Attenuation of the neural response to sad faces in major depression by antidepressant treatment—a prospective, event-related functional magnetic resonance imaging study. Arch. Gen. Psychiatry 61, 877–889.

Genovese, C.R., Lazar, N.A., Nichols, T., 2002. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. NeuroImage 15, 870–878.

Grabe, H.J., Lange, M., Wolff, B., Volzke, H., Lucht, M., Freyberger, H.J., John, U., Cascorbi, I., 2005. Mental and physical distress is modulated by a polymorphism in the 5-HT transporter gene interacting with social stressors and chronic disease burden. Mol. Psychiatry 10, 220–224.

Gur, R.C., Schroeder, L., Turner, T., McGrath, C., Chan, R.M., Turetsky, B.I., Alsop, D., Maldjian, J., Gur, R.E., 2002. Brain activation during facial emotion processing. NeuroImage 16, 651–662.

Hariri, A.R., Mattay, V.S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., Egan, M.F., Weinberger, D.R., 2002. Serotonin transporter genetic variation and the response of the human amygdala. Science 297, 400–403.

Hariri, A.R., Drabant, E.M., Munoz, K.E., Kolachana, L.S., Mattay, V.S., Egan, M.F., Weinberger, D.R., 2005. A susceptibility gene for affective disorders and the response of the human amygdala. Arch. Gen. Psychiatry 62, 146–152.

Heinz, A., Smolka, M.N., Braus, D.F., Wrase, J., Beck, A., Flor, H., Mann, K., Schumann, G., Buchel, C., Hariri, A.R., Weinberger, D.R., 2007. Serotonin transporter genotype 5-HTTLPR: Effects of neutral and undefined conditions on amygdala activation. Biol. Psychiatry 61, 1011–1014.

Iidaka, T., Ozaki, N., Matsumoto, A., Nogawa, J., Kinoshita, Y., Suzuki, T., Iwata, N., Yamamoto, Y., Okada, T., Sadato, N., 2005. A variant C178T in the regulatory region of the serotonin receptor gene HTR3A modulates neural activation in the human amygdala. J. Neurosci. 25, 6460–6466.

Kaufman, J., Yang, B.Z., Douglas-Palumberi, H., Grasso, D., Lipschitz, D., Houshyar, S., Krystal, J.H., Gelernter, J., 2006. Brain-derived neurotrophic factor–5-HTTLPR gene interactions and environmental modifiers of depression in children. Biol. Psychiatry 59, 673–680.

Kendler, K.S., Prescott, C.A., 1999. A population-based twin study of lifetime major depression in men and women. Arch. Gen. Psychiatry 56, 39–44.

Kendler, K.S., Heath, A., Martin, N.G., Eaves, L.J., 1986. Symptoms of anxiety and depression in a volunteer twin population—the etiologic role of genetic and environmental-factors. Arch. Gen. Psychiatry 43, 213–221.

Kumari, V., Mitterschiffthaler, M.T., Teasdale, J.D., Malhi, G.S., Brown, R.G., Giampietro, V., Brammer, M.J., Poon, L., Simmons, A., Williams, S.C.R., Checkley, S.A., Sharma, T., 2003. Neural abnormalities during cognitive generation of affect in treatment-resistant depression. Biol. Psychiatry 54, 777–791.

Lawrence, N.S., Williams, A.M., Surguladze, S., Giampietro, V., Brammer, M.J., Andrew, C., Frangou, S., Ecker, C., Phillips, M.L., 2004. Subcortical and ventral prefrontal cortical neural responses to facial expressions distinguish patients with bipolar disorder and major depression. Biol. Psychiatry 55, 578–587.

Mackintosh, M.A., Gatz, M., Wetherell, J.L., Pedersen, N.L., 2006. A twin study of lifetime Generalized Anxiety Disorder (GAD) in older adults: genetic and environmental influences shared by neuroticism and GAD. Twin Res. Hum. Genet. 9, 30–37.

Martin, N., Boomsma, D., Machin, G., 1997. A twin-pronged attack on complex traits. Nat. Genet. 17, 387–392.

Middeldorp, C.M., Cath, D.C., Beem, A.L., Boomsma, D.I., 2004. Genetic epidemiology of depression in a selected population of Dutch twins and their siblings. Behav. Genet. 34, 652–653.

Montgomery, S.A., Asberg, M., 1979. New depression scale designed to be sensitive to change. Br. J. Psychiatry 134, 382–389.

Munafò, M.R., Brown, S.M., Hariri, A.R., 2007. Serotonin transporter (5-HTTLPR) genotype and amygdala activation: a meta-analysis. Biol. Psychiatry (Oct 17, Electronic publication ahead of print).

Peters, L., Andrews, G., 1995. Procedural validity of the computerized version of the Composite International Diagnostic Interview Cidi-Auto in the Anxiety Disorders. Psychol. Med. 25, 1269–1280.

Pezawas, L., Angst, J., Kasper, S., 2005. Recurrent brief depression revisited. Int. Rev. Psychiatry 17, 63–70.

Phillips, M.L., Drevets, W.C., Rauch, S.L., Lane, R., 2003. Neurobiology of emotion perception: II. Implications for major psychiatric disorders. Biol. Psychiatry 54, 515–528.

Rauch, S.L., Whalen, P.J., Shin, L.M., McInerney, S.C., Macklin, M.L., Lasko, N.B., Orr, S.P., Pitman, R.K., 2000. Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. Biol. Psychiatry 47, 769–776.

Schienle, A., Schafer, A., Stark, R., Walter, B., Vaitl, D., 2005. Gender differences in the processing of disgust- and fear-inducing pictures: an fMRI study. NeuroReport 16, 277–280.

Shekhar, A., Sajdyk, T.J., Gehlert, D.R., Rainnie, D.G., 2003. The amygdala, panic disorder, and cardiovascular responses. Amygdala in Brain Function: Basic and Clinical Approaches, vol. 985, pp. 308–325.

Sheline, Y.I., Barch, D.M., Donnelly, J.M., Ollinger, J.M., Snyder, A.Z., Mintun, M.A., 2001. Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. Biol. Psychiatry 50, 651–658.

Shin, L.M., Wright, C.I., Cannistraro, P.A., Wedig, M.M., McMullin, K., Martis, B., Macklin, M.L., Lasko, N.B., Cavanagh, S.R., Krangel, T.S., Orr, S.P., Pitman, R.K., Whalen, P.J., Rauch, S.L., 2005. A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. Arch. Gen. Psychiatry 62, 273–281.

Siegle, G.J., Granholm, E., Ingram, R.E., Matt, G.E., 2001. Pupillary and reaction time measures of sustained processing of negative information in depression. Biol. Psychiatry 49, 624–636.

Spielberger, C.D., Gorsuch, R.L., Lushene, R.E., 1970. STAI Manual for the State-Trait Anxiety Inventory. Consulting Psychologists Press, Palo Alto, CA.

Stark, C.E.L., Squire, L.R., 2001. When zero is not zero: the problem of ambiguous baseline conditions in fMRI. Proc. Natl. Acad. Sci. U. S. A. 98, 12760–12765.

Stein, M.B., Goldin, P.R., Sareen, J., Zorrilla, L.T.E., Brown, G.G., 2002. Increased amygdala activation to angry and contemptuous faces in generalized social phobia. Arch. Gen. Psychiatry 59, 1027–1034.

Sullivan, P.F., Neale, M.C., Kendler, K.S., 2000. Genetic epidemiology of major depression: review and meta-analysis. Am. J. Psychiatry 157, 1552–1562.

Surguladze, S., Brammer, M.J., Keedwell, P., Giampietro, V., Young, A.W., Travis, M.J., Williams, S.C.R., Phillips, M.L., 2005. A differential pattern of neural response toward sad versus happy facial expressions in major depressive disorder. Biol. Psychiatry 57, 201–209.

Thomas, K.M., Drevets, W.C., Dahl, R.E., Ryan, N.D., Birmaher, B., Eccard, C.H., Axelson, D., Whalen, P.J., Casey, B.J., 2001. Amygdala response to fearful faces in anxious and depressed children. Arch. Gen. Psychiatry 58, 1057–1063.

Wechsler, D., 1997. WAIS-III Wechsler Adult Intelligence Scale. Psychological Corporation, San Antonio, Texas.

Wittchen, H.U., 1994. Reliability and validity studies of the Who Composite International Diagnostic Interview Cidi—a critical-review. J. Psychiat. Res. 28, 57–84.

Wright, C.I., Martis, B., McMullin, K., Shin, L.M., Rauch, S.L., 2003. Amygdala and insular responses to emotionally valenced human faces in small animal specific phobia. Biol. Psychiatry 54, 1067–1076.

Yang, T.T., Menon, V., Eliez, S., Blasey, C., White, C.D., Reid, A.J., Gotlib, I.H., Reiss, A.L., 2002. Amygdalar activation associated with positive and negative facial expressions. NeuroReport 13, 1737–1741.