Psychological Medicine (2015), **45**, 1447–1458. © Cambridge University Press 2014
doi:10.1017/S0033291714002542

ORIGINAL ARTICLE

Different neural and cognitive response to emotional faces in healthy monozygotic twins at risk of depression

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Background. Negative cognitive bias and aberrant neural processing of emotional faces are trait-marks of depression. Yet it is unclear whether these changes constitute an endophenotype for depression and are also present in healthy individuals with hereditary risk for depression.

Method. Thirty healthy, never-depressed monozygotic (MZ) twins with a co-twin history of depression (high risk group: $n = 13$) or without co-twin history of depression (low-risk group: $n = 17$) were enrolled in a functional magnetic resonance imaging (fMRI) study. During fMRI, participants viewed fearful and happy faces while performing a gender discrimination task. After the scan, they were given a faces dot-probe task, a facial expression recognition task and questionnaires assessing mood, personality traits and coping strategies.

Results. High-risk twins showed increased neural response to happy and fearful faces in dorsal anterior cingulate cortex (ACC), dorsomedial prefrontal cortex (dmPFC), pre-supplementary motor area and occipito-parietal regions compared to low-risk twins. They also displayed stronger negative coupling between amygdala and pregenual ACC, dmPFC and temporo-parietal regions during emotional face processing. These task-related changes in neural responses in high-risk twins were accompanied by impaired gender discrimination performance during face processing. They also displayed increased attention vigilance for fearful faces and were slower at recognizing facial expressions relative to low-risk controls. These effects occurred in the absence of differences between groups in mood, subjective state or coping.

Conclusions. Different neural response and functional connectivity within fronto-limbic and occipito-parietal regions during emotional face processing and enhanced fear vigilance may be key endophenotypes for depression.

Received 30 April 2014; Revised 25 September 2014; Accepted 25 September 2014; First published online 10 November 2014

Key words: Depression, emotional processing, endophenotype, fMRI, risk, twins.

Introduction

Limited success in the search for susceptibility genes in major depression has increased the interest in identifying endophenotypes for depression (Gottesman & Gould, 2003; Hasler *et al.* 2004; Hasler & Northoff, 2011). Endophenotypes are highly heritable disease-associated traits that are specific to the illness, expressed independently of the clinical state and are present in non-affected family members (Gershon & Goldin, 1986; Goldin *et al.* 1986; Gottesman & Gould, 2003). Discovery of endophenotypes may offer new insights into the aetiology of depression and aid earlier detection and correct treatment (Hasler *et al.* 2004; Hasler & Northoff, 2011).

Compelling evidence points to negative cognitive bias and altered neural processing of emotional faces as potential endophenotypes for depression. Depressed patients show selective attention and vigilance to negative (fearful and/or sad) faces, reduced attention to positive (happy) expressions (Suslow *et al.* 2001; Gotlib *et al.* 2004; Le *et al.* 2007; Leyman *et al.* 2007), and reduced recognition of positive expressions and/or increased recognition of negative emotions (Feinberg *et al.* 1986; Persad & Polivy, 1993; Asthana *et al.* 1998). Patients also display a bias towards interpreting neutral or ambiguous expressions as negative (Bouhuys *et al.* 1996, 1999; Hale, 1998; Hale *et al.* 1998; Leppanen *et al.* 2004) which predicts illness persistence and relapse (Hale, 1998; Bouhuys *et al.* 1999).

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Functional neuroimaging studies have linked this negative bias to increased neural response to negative faces and decreased response to positive faces in the amygdala, fusiform gyrus, parahippocampal gyrus, insula, and putamen (for review see Stuhrmann *et al.* 2011). Negative faces also produce hyper-activation in the motor cortex, middle and subgenual anterior cingulate cortex (ACC) and hypo-activation in the medial, inferior and dorsolateral prefrontal cortex (dlPFC) in depressed patients, whereas evidence regarding the direction of PFC activation to positive expressions is less consistent (Stuhrmann *et al.* 2011). These findings and the presence of reciprocal inhibitory amygdala–PFC connections have been integrated in a model of emotional processing in depression which attributes negative cognitive bias in depression to an over-activity in regions involved in initial evaluation of emotional stimuli and defective prefrontal top-down control (Phillips *et al.* 2003). In keeping with this model, functional connectivity, which reflects correlation of activity over time between brain regions, is reduced between amygdala and prefrontal regions during negative emotional processing in depressed patients (Anand *et al.* 2005*a*; Chen *et al.* 2007; Dannlowski *et al.* 2009; Erk *et al.* 2010; Kong *et al.* 2013) and is reversed by successful antidepressant drug treatment (Anand *et al.* 2005*b*; Chen *et al.* 2008; Ruhe *et al.* 2012).

Negative face processing bias also fulfils the endophenotype criterion of being expressed independently of the clinical state. Recovered depressed patients display increased attention to and recognition of negative facial expressions (Bhagwagar *et al.* 2004; Joormann & Gotlib, 2007; LeMoult *et al.* 2009) and tend to interpret ambiguous facial expressions as negative (Bouhuys *et al.* 1999). This is paired with an increased neural response of dlPFC)and caudate during supraliminal processing of fearful faces (Norbury *et al.* 2010; Thomas *et al.* 2011) and increased responsiveness of the amygdala during subliminal processing of negative faces (Neumeister *et al.* 2006; Victor *et al.* 2012). Remitted patients also show more negative functional connectivity (coupling) between amygdala and PFC during processing of sad faces (Goulden *et al.* 2012). Together these neuroimaging findings suggest that recovered patients display greater pre-conscious vigilance to negative faces paired with increased compensatory top-down control of limbic reactivity during conscious processing of negative expressions.

Relatively few studies have investigated whether negative face processing bias fulfils the endophenotype criterion of being expressed in non-affected family members. One study of healthy individuals with first-degree family history of depression reported faster recognition of fearful expressions (Le *et al.* 2007), but another study in young unaffected individuals with a first-degree relative with depression found no such bias (Mannie *et al.* 2007). It is possible that homoeostatic mechanism in healthy individuals counteracts the behavioural expression of aberrant neural responses to emotional face stimuli. Indeed, healthy individuals with a first-degree relative with depression displayed an attenuated neural response in the dlPFC to fearful faces (Mannie *et al.* 2011). This prefrontal hypo-activity was not associated with differences in amygdala responses (Mannie *et al.* 2011), although amygdala hyper-activity to fearful faces was demonstrated in at-risk individuals during a passive face viewing condition (Monk *et al.* 2008). These findings may provide a neural correlate for the negative face processing bias. Nevertheless, the evidence is scarce and there is an acute lack of studies of functional connectivity during emotional face processing in individuals at familial risk for depression.

Studies on healthy monozygotic (MZ) twins with or without co-twin history of depression provide a strong methodology for research into endophenotypes since MZ twins are (nearly always) genetically identical and have high concordance rates of 23–67% (Kendler *et al.* 1993). Only one study has investigated emotional face processing in MZ twins at high *v.* low risk for depression (Wolfensberger *et al.* 2008) and reported increased amygdala response to negative faces in high-risk *v.* low-twins. However, risk status was defined according to participants' scores of anxiety, neuroticism and depression and no assessment was made of potential history of depression. It is therefore possible that this finding reflected effects of mood or previous depression. We have demonstrated mild cognitive deficits in healthy MZ and dizygotic (DZ) twins at heritable risk for depression (Christensen *et al.* 2006). However, it is unclear whether these at-risk individuals also show negative face processing bias. In the current study, we therefore investigated neural and cognitive response to emotional faces in healthy, never-depressed MZ twins with or without a co-twin history of depression (high-risk *v.* low-risk groups). We hypothesized that compared to low-risk twins, high-risk twins would display a negative cognitive bias in the processing of facial expressions along with abnormalities in limbic and fronto-parietal response to negative *v.* positive faces that resemble the alterations found in depression.

Method and materials

Participants and recruitment

Thirty healthy, never-depressed MZ twins were enrolled in the study as a part of an ongoing high-risk

https://doi.org/10.1017/S0033291714002542 Published online by Cambridge University Press

study elucidating risk factors for affective disorder in a large cohort of high- and low-risk twins ($N = 234$) approved by the Danish Ministry of Healthy, Danish Scientific Ethics Committee, and the Data Protection Agency (for details, see Vinberg *et al.* 2013). This cohort was originally identified and recruited through record linkage between the nationwide Danish Twin Registry, the Danish Psychiatric Central Research Register and the Danish Civil Registration System and participated in a cross-sectional baseline study in 2003–2005 (39;41). The twins were then followed for a period of 7 years during which time they completed the 21-item Beck Depression Inventory (BDI-21) and the Mood Disorder Questionnaire (MDQ) sent to them by post at 6-month intervals followed by telephone interviews in 2010–2012, on which occasion they were asked to participate in the present functional magnetic resonance imaging (fMRI) study. Thirteen MZ twins had a co-twin history of hospital admission for major depression (high-risk group), whereas the remaining 17 MZ twins had no co-twin or first-degree family history of psychiatric illness including depression (low-risk group). Inclusion criteria for this study were no personal history of psychiatric or organic brain illness at any assessment time.

Experimental design

Participants were investigated at the Danish Research Center for Magnetic Resonance (DRCMR) between 12:00 and 21:00 hours. They started by completing a set of questionnaires for measurement of their mood and subjective state, personality traits, and coping style which was followed by fMRI assessing neural response to emotional faces. After the scan participants completed a faces dot-probe and a facial expression recognition task on a test computer. The experimenters were blinded to group membership and blinding was maintained throughout data management and analysis.

Emotional face processing task during fMRI

Neural response to emotional faces was assessed with fMRI while participants performed a faces processing task from the Emotional Test Battery (ETB) using the NimStim Face Stimulus Set (http://www.macbrain.org/resources.htm). Pictures of happy or fearful faces were projected from a computer using E-Prime software version 1.2 (Psychology Software Tools Inc., USA) onto an opaque screen at the foot end of the scanner bed, which could be seen by the participants through an angled mirror. Happy and fearful faces were presented in a block paradigm. Each block lasted 25 s and consisted of 10 pictures of happy or fearful faces each shown on the screen for 200 ms immediately followed by a fixation cross shown for 2300 ms. Blocks were interleaved by 16 s inter-blocks with a central fixation cross. There were four blocks of each emotion condition and eight inter-blocks, leading to a total task time of 5 min 28 s. The participants were instructed to perform a gender discrimination task by pressing the keys of a response pad with their right middle and index fingers for male and female, respectively, as quickly and accurately as possible. Participants' responses were recorded and used for the calculation of mean reaction times (RT) and task accuracy.

Behavioural tasks outside the scanner

Faces dot-probe task

Vigilance to happy and fearful facial expressions was investigated with a faces dot-probe task from the ETB, in which pairs of faces were presented on the computer screen. Each pair consisted of an emotional and a neutral expression or two neutral expressions of the same person. There were an equal number of three types of face pairs: happy-neutral, fearful-neutral and neutral-neutral. The trails were divided into masked and unmasked conditions. In the unmasked emotion condition face pairs were shown for 100 ms, and in the masked condition the emotional faces were shown for 17 ms immediately replaced by a neutral mask for 83 ms. In each trial one face was immediately replaced by two dots presented either vertically (:) or horizontally (· ·). Participants were instructed to indicate the orientation of the dots as quickly and accurately as possible by pressing labelled keys on the keyboard. The dots remained on the screen until participants had made their response. There was a total of 192 trials divided into 32 masked happy-neutral pairs, 32 masked fear-neutral pairs, 32 masked neutral-neutral pairs, 32 unmasked happy-neutral pairs, 32 unmasked fear-neutral pairs and 32 unmasked neutral-neutral pairs. Eight blocks of unmasked trials (12 trials per block) and eight blocks of masked trials (12 trials per block) were presented in an alternating order with an ABAB design (for more details see Murphy *et al.* 2008). RT for correct responses and accuracy were recorded.

Facial expression recognition task

In the facial expression recognition task from the ETB participants viewed pictures of faces expressing one of the six basic emotions: happiness, surprise, sadness, fear, anger and disgust. Pictures of emotional faces were taken from Ekman & Friesen (1979) and were presented on the screen of a laptop computer in randomized order. Each face was presented for 500 ms immediately replaced by a blank screen. The

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participants were instructed to determine the particular emotional expression as quickly and accurately as possible by pressing the corresponding key on the keyboard. The emotional faces were morphed at 10% steps in shape and texture differences between a neutral face (0%) and a full emotion face (100%) (for more details see Harmer *et al.* 2004). There were a total of 250 stimuli presentations consisting of four examples of every emotion at each intensity level plus a neutral face for every emotion. RT for correct responses, accuracy and misclassifications were recorded.

Mood and subjective state

Mood and subjective state were assessed with the State and Trait Anxiety Inventory (STAI), the Beck Depression Inventory (BDI; Beck *et al.* 1961) and visual analogue scales (VAS) of relevant mood states (happiness, sadness, arousal, anxiety, dizziness, nausea). Neuroticism was assessed with the Eysenck Personality Questionnaire (EPQ; Beck *et al.* 1961; Eysenck & Eysenck, 1975), and coping styles measured with the Coping Index for Stressful Situations (CISS; Beck *et al.* 1961; Endler & Parker, 1990).

Magnetic resonance imaging

MRI data were collected at the DRCMR with a 3 T Siemens Trio MR scanner using an eight-channel head array coil. Blood oxygen-level dependent (BOLD)-sensitive fMRI used a T2*-weighted gradient echo spiral echo-planar imaging (EPI) sequence with an echo time (TE) of 30 ms, repetition time (TR) of 2.49 ms and a low flip angle of 20° to minimize physiological noise (Gonzalez-Castillo *et al.* 2011). A total of 128 brain volumes were acquired in a single fMRI session, each consisting of 42 slices with a slice thickness of 3 mm and a field of view (FOV) of 192 × 192 mm using a 64 × 64 grid. High-resolution 3D structural T1-weighted spin echo images were obtained after the first session of BOLD fMRI (TI = 800, TE = 3.93, TR = 1540 ms, flip angle 9°; 256 × 256 FOV; 192 slices).

fMRI data analysis

fMRI data was pre-processed and analysed using the software tools within FMRIB Software Library version (FSL version 4.1) (www.fmrib.ox.ac.uk/fsl; Smith *et al.* 2004). Pre-processing included image realignment, non-brain removal, spatial normalization and spatially smoothing (Gaussian kernel, 5 mm full-width-half-maximum). The time series in each session were high pass-filtered (to a maximum of 0.008 Hz). Analyses of individual subject data were computed using the general linear model with local autocorrelation correction (Woolrich *et al.* 2001). Two experimental conditions – 'fearful faces' and 'happy faces' – were modelled separately by convolving trials with a canonical hemodynamic response function (Boynton *et al.* 1996). At the group level, all analyses employed a full mixed-effects approach (Woolrich *et al.* 2004). *Z* (Gaussian *T*) statistic images were thresholded using clusters determined by $Z > 2.0$ and a cluster significance of $p < 0.05$ corrected for multiple comparison at a cluster level. A standard anatomical atlas (Talairach & Tournoux, 1988) was used to localize the foci of peak cluster activation. Following face contrasts were chosen: fearful > happy, happy > fearful, fearful and happy > baseline. Mean per cent BOLD signal change was extracted and examined with analysis of variance (ANOVA) for clusters which were activated during these contrasts in the low-risk control group (main effect of task) and for clusters which showed significant between-group interactions in the whole-brain analysis.

To assess amygdala response to emotional faces, regions of interest (ROIs) for the left and right amygdala in standard space were obtained with mri3dX (http://www.aston.ac.uk/lhs/staff/singhkd/mri3dX/mri3dX.jsp), which uses a stored representation of the Talairach Daemon Database (Lancaster *et al.* 2000). Mean per cent BOLD signal change to fearful and happy faces was computed in left and right amygdala separately and compared between the groups. Mean per cent signal change was also extracted from a functional mask within the left and right amygdala, respectively (i.e. amygdala clusters responding specifically to fearful *v.* happy faces across all participants) obtained by using the amygdala structural mask for small volume correction (SVC) in FSL (thresholded at $Z > 2.0$ and $p < 0.05$, corrected for multiple comparisons at a cluster level).

Functional connectivity analysis was performed by extracting for each participant a deconvolved time series for (*a*) the emotional face blocks *v.* baseline cluster identified within the anatomical right amygdala using SVC and (*b*) the functional cluster within the anatomical left amygdala. These time-courses were then entered in two separate FSL psychophysical interaction (PPI) analyses with the functional right amygdala cluster and the functional left amygdala cluster as the seed region, respectively, along with the two psychological regressors (fear, happy) and the two PPI regressors (fear × time series, happy × time series). These individual contrast images were then entered into the group level (high-risk *v.* low-risk group) using a mixed-effects analysis across the whole brain to identify brain areas in which regional activity co-varied stronger with that of the left and right amygdala in one of the two groups during fear blocks, happy blocks, and emotional face blocks in general. *Z* statistic images were thresholded at $Z > 2.0$, with a

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Table 1. *Demographic information and mood ratings on the test day for high-risk and control groups*

	High-risk ($n = 13$)	Controls ($n = 17$)	p value
Age, years, mean (S.D.)	48.6 (14.7)	42.5 (10.2)	0.19
Gender, no. female (%)	8 (62%)	11 (65%)	0.86
Years of education, mean (S.D.)	15.8 (3.3)	16.3 (2.6)	0.66
Neuroticism	3.1 (2.8)	4.5 (4.1)	0.31
Coping style			
Task-oriented	33.5 (5.8)	37.5 (13.9)	0.34
Emotion-oriented	43.7 (5.8)	43.4 (11.8)	0.93
Avoidance-oriented	45.2 (4.5)	45.1 (5.4)	0.99
BDI, mean (S.D.)	1.4 (2.1)	1.2 (1.8)	0.84
STAI-state, mean (S.D.)	28.3 (4.7)	27.2 (5.5)	0.58
STAI-trait, mean (S.D.)	27.2 (4.5)	28.9 (7.2)	0.46
VAS of subjective state			
Happiness, mean (S.D.)	63.5 (11.9)	66.3 (12.7)	0.53
Sadness, mean (S.D.)	9.9 (16.0)	12.7 (10.8)	0.57
Alertness, mean (S.D.)	68.6 (10.7)	65.5 (20.3)	0.62
Anxiety, mean (S.D.)	7.2 (16.1)	8.1 (14.7)	0.88
Dizziness, mean (S.D.)	7.8 (9.7)	4.3 (7.3)	0.27
Nausea, mean (S.D.)	4.7 (8.9)	5.5 (11.9)	0.84

BDI, Beck Depression Inventory; STAI, State and Trait Anxiety Inventory; VAS, Visual Analogue Scale.

cluster threshold of $p < 0.05$, including a multiple-comparison correction. In addition, we tested for a linear relationship between significant clusters of differential connectivity between the groups and fear vigilance in the dot-probe test and state-trait anxiety scores with Pearson's correlation analyses for PPI standardized betas *v.* fear vigilance/ anxiety scores.

Statistical analysis of behavioural and mood data

Mood ratings and behavioural data were analysed using repeated-measures ANOVA with group as the between-subjects factor. Simple main effect analyses were used for further analysis for significant interactions. Signal detection theory was applied to obtain a measure of accuracy for facial expression recognition corrected for the participants' response tendency (d') (Grier, 1971). All statistical analyses were performed with SPSS software v. 15.0 (SPSS Inc., USA).

Results

Participant characteristics and mood

Table 1 displays demographic information, mood and subjective state, neuroticism and coping scores for the high-risk and low-risk group. Groups were matched for age, gender and education levels (p values > 0.19) and showed no differences in mood and subjective state (p values > 0.27), neuroticism ($p > 0.31$) or coping styles (p values > 0.34) (see Table 1).

fMRI results

Whole-brain analysis

Fearful and happy faces *v.* baseline activated a broad network of prefrontal and occipito-parietal regions in the low-risk group in keeping with previous findings (see Fig. 1; for peak cluster activations see Table 2). Comparison of the extracted mean per cent BOLD signal change within each of the clusters in this face processing network showed no differential response to fearful and happy expressions between groups (p values > 0.38).

Exploratory whole-brain analysis revealed greater neural response to both fearful and happy faces in high-risk *v.* low-risk twins within a network including the caudal and dorsal ACC, the posterior part of the dorsomedial prefrontal cortex (dmPFC), pre-supplementary motor area (pre-SMA), the superior temporal gyrus, the inferior and superior parietal cortex including the precuneus, and the fusiform gyrus ($F_{1,28} = 20.60$, $p < 0.0001$) (see Fig. 1; for peak cluster activations see Table 2). Neural response to fearful *v.* happy faces showed no differences between groups.

Amygdala ROI

Consistent with previous studies, fearful and happy faces produced significant left and right amygdala activation (fearful: p values < 0.002; happy: p values < 0.04). High- and low-risk groups showed no difference in amygdala response to fearful or happy faces

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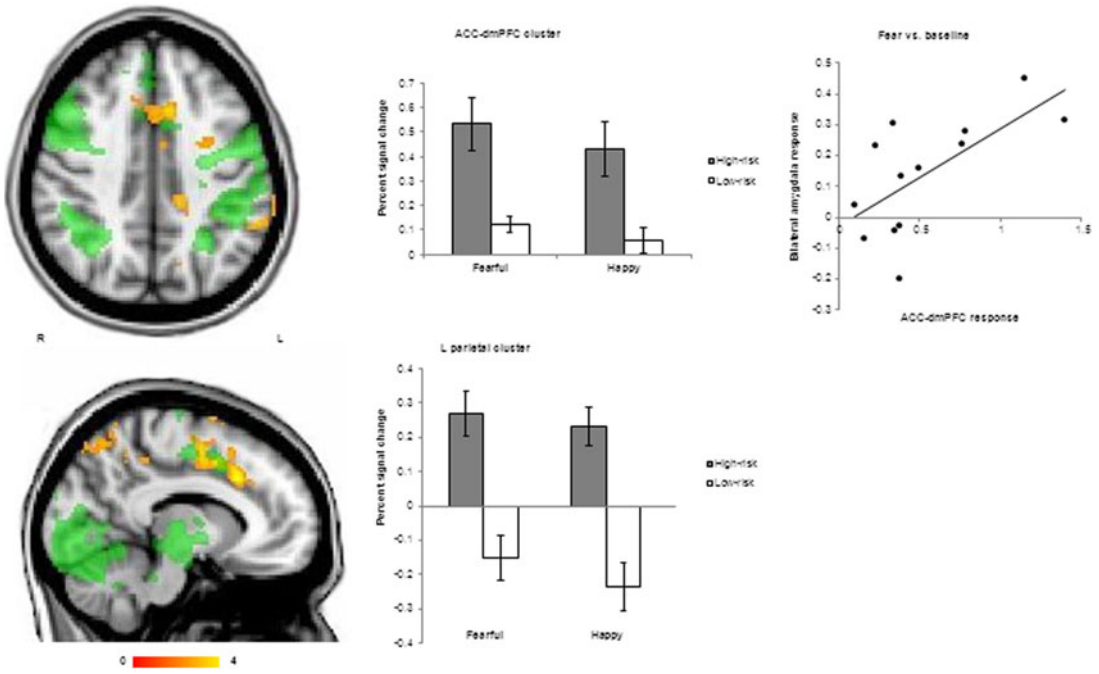

Fig. 1. Neural response to emotional faces (happy and fearful) *v.* baseline in low-risk MZ twins (main effect of task; marked with green) and areas showing a stronger response to emotional faces in high-risk relative to low-risk twins (group × task interaction; marked with yellow). Extraction of blood oxygen-level dependent (BOLD) signal change from the regions showing a group × task interaction revealed that high-risk twins ($N = 13$) displayed increased BOLD signal change compared to controls ($N = 17$) to both fearful and happy faces within the anterior cingulate cortex-dorsomedial prefrontal cortex (ACC-dmPFC) and left parietal cortex. Values represent mean percentage signal change. Error bars represent the S.E.M. In high-risk twins, ACC-posterior dmPFC response correlated positively with bilateral amygdala response to fearful faces *v.* baseline.

(p values > 0.17 and p values > 0.41, respectively). *Post-hoc* Pearson's correlation analysis in the high-risk group revealed a positive correlation between per cent signal change in bilateral amygdala response and in the posterior dmPFC-ACC during processing of fearful but not happy faces (Fig. 1, Table 2) ($r_{11} = 0.66$, $p = 0.02$).

Right and left amygdala whole-brain PPI results

Whole-brain PPI analysis with the right and left amygdala functional cluster (SVC; emotional faces *v.* baseline in controls) as the seed regions revealed more negative coupling between right amygdala and pregenual ACC during emotional face blocks (*v.* baseline) in high-risk *v.* low-risk twins (see Fig. 2; for peak of negative coupling see Table 2). High-risk twins also showed more negative left amygdala coupling with activity in pregenual ACC, posterior dmPFC, posterior cingulate cortex (PCC), and bilateral temporo-parietal regions during emotional face blocks (see Fig. 2; for peaks of negative coupling within these clusters see Table 2).

Across both groups, the individual increase in negative connectivity between right amygdala and pregenual ACC and between left amygdala and dmPFC and posterior cingulate was associated with greater fear vigilance (right amygdala-pregenual ACC: $r_{28} = -0.39$, $p = 0.034$; left amygdala-dmPFC: $r_{28} = -0.37$, $p = 0.046$; left amygdala-PCC: $r_{28} = -0.37$, $p = 0.043$) (Fig. 2). No such association was found for the other clusters (all p values > 0.06). There was no correlation between connectivity from amygdala and state or trait anxiety (p values > 0.09).

Behavioural results

Gender discrimination during scanning

Gender discrimination data for one low-risk twin was not acquired due to technical difficulties; analysis therefore included 13 high-risk and 16 low-risk twins. High-risk twins showed reduced gender discrimination accuracy for both fearful and happy faces (fear: $t = 1.99$, df = 14.0, $p = 0.04$; happy: $t = 2.3$, df = 13.0, $p = 0.02$) in the absence of RT differences (p values > 0.61) (Fig. 3*a*). Gender discrimination accuracy correlated negatively with left amygdala response to emotional faces in the high-risk group ($r_{11} = -0.64$, $p = 0.02$) but not in low-risk controls ($p > 0.35$). In

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Table 2. *Peak cluster activation in brain regions of increased BOLD response (whole-brain analyses with Z = 2.0, p = 0.05, cluster-corrected) during processing of fearful and happy faces v. fixation baseline in low-risk MZ twins (main effect of task) and in high-risk twins v. low-risk twins (high-risk > low-risk) as well as peaks in clusters showing increased negative functional connectivity with amygdala in high-risk v. low-risk group (high-risk > low-risk) in whole-brain PPI analyses (Z = 2.0, p = 0.05, cluster-corrected)*

		Coordinates		
Task and region	Z value	x	y	z
Emotional faces *v.* fixation baseline				
Main effect of task				
Right fusiform gyrus (BA 37)	6.29	46	−52	−22
Left medial frontal cortex (BA 6)	4.51	−6	−12	52
Right inferior parietal cortex (BA 40)	4.26	46	−42	38
High-risk MZ twins>low-risk MZ twins				
Left superior frontal gyrus (BA 6)	5.53	−24	−8	72
Left inferior frontal gyrus (BA 45)	4.30	−60	26	18
Right superior frontal gyrus (BA 6)	3.29	18	−18	78
Left inferior parietal gyrus (BA 40)	4.01	−66	−30	32
Left fusiform gyrus (BA 19)	3.61	−22	−64	−20
Negative functional connectivity from amygdala, high-risk > controls				
Right amygdala functional cluster				
Left medial frontal gyrus (BA 10)	3.13	−2	60	6
Left amygdala functional cluster				
Right superior frontal gyrus (BA 6)	3.14	6	18	62
Left anterior cingulate (BA 32)	3.46	−2	44	−6
Left superior temporal gyrus (BA 22)	3.43	−64	−48	16
Left posterior cingulate (BA 29)	3.28	−4	−56	6
Right middle temporal gyrus (BA 39)	3.50	46	−66	20
Left posterior cingulate (BA 23)	3.43	0	−48	20

BOLD, Blood oxygen-level dependent; PPI, psychophysical interaction; BA, Brodmann area.
MNI coordinates (x, y, z) refer to the point of peak activation within each cluster identified using this threshold.

contrast, neural response in the regions of increased activity to emotional faces in high-risk *v.* low-risk twins (Fig. 1, Table 2) showed no correlation with gender discrimination accuracy in this group (p values < 0.24).

Faces dot-probe

High-risk twins displayed increased subliminal vigilance towards fearful faces relative to low-risk twins, as reflected by higher accuracy for determination of orientation when these replaced masked fearful *v.* neutral faces ($t = 2.63$, df = 28, $p = 0.01$) in the absence of differences in RTs (p values > 0.27) (Fig. 3*b*). This effect occurred in the absence of between-group differences in vigilance to masked happy faces or to unmasked (consciously processed) happy or fearful faces (accuracy: p values > 0.65; RT: p values > 0.47).

Facial expression recognition

Accuracy and RT revealed no negative bias within facial expression recognition in high-risk *v.* low-risk twins (p values > 0.14). However, the high-risk group displayed increased response latency during recognition of facial expressions in general relative to those at low risk ($F_{1,28} = 5.48$, $p = 0.03$) (Fig. 3*c*). This effect occurred in the absence of differences between groups in overall recognition accuracy (p values > 0.29).

Discussion

This study is the first to investigate neural and cognitive response to emotional faces in healthy, never-depressed MZ twins at high *v.* low familial risk for depression. High-risk twins showed exaggerated neural response to both fearful and happy faces in the caudal and dorsal ACC, posterior dmPFC, pre-SMA, and occipito-parietal regions. They also displayed more negative connectivity between amygdala and pregenual ACC, dmPFC and temporo-parietal regions relative to low-risk twins. At a behavioural level this was accompanied by impaired gender discrimination performance during face processing, enhanced subliminal vigilance towards fearful faces and increased response latencies during facial

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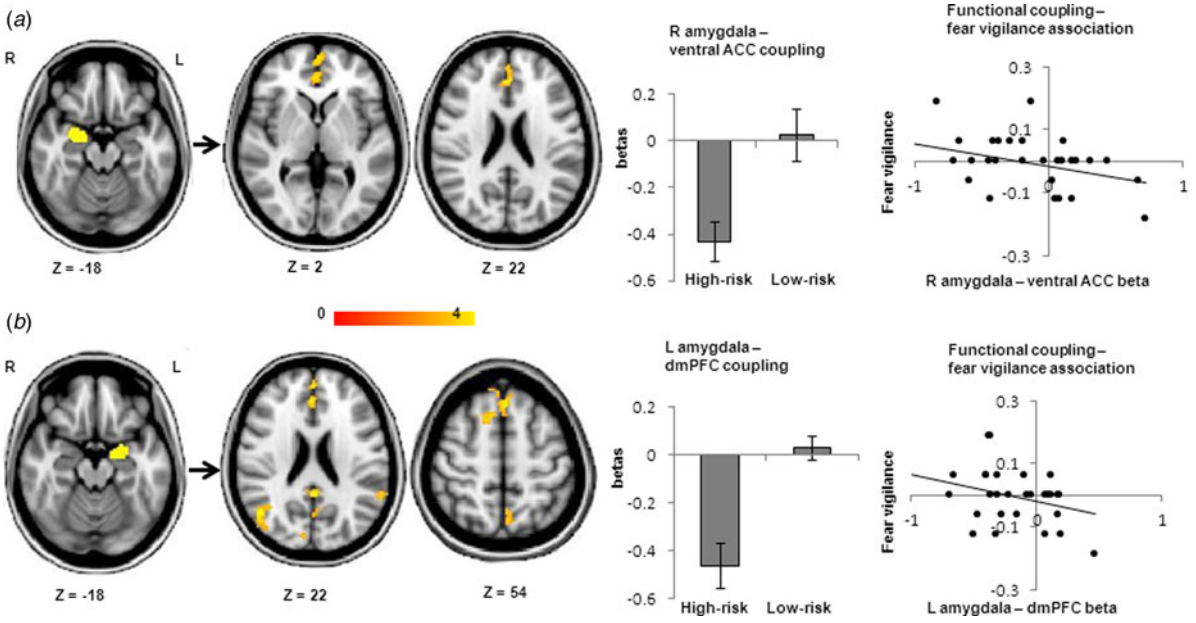

Fig. 2. Whole-brain psychophysical interaction analysis with right amygdala (*a*) and left amygdala (*b*) functional cluster (emotional faces *v.* baseline in controls) as the seed region: high-risk twins showed increased negative connectivity of the right amygdala with pregenual anterior cingulate cortex (ACC) and of the left amygdala with pregenual ACC, dorsomedial prefrontal cortex (dmPFC), left temporo-parietal regions and right occipital cortex. Negative coupling between between right amygdala seed and pregenual ACC and between left amygdala seed and dmPFC correlated with subliminal fear vigilance.

expression recognition. These effects occurred in the absence of differences between groups in mood, subjective state or coping styles.

The exaggerated occipito-parietal response to both happy and fearful faces in high-risk twins contrasts with our negative bias hypothesis when considered alone. However, across all participants increased occipito-parietal responses to fearful faces was associated with greater subliminal fear vigilance, consistent with the particular engagement of these regions in early perceptual and attentional processing of threat (Pourtois & Vuilleumier, 2006). We therefore suggest that the exaggerated occipito-parietal response to both fearful and happy faces in high-risk twins could indicate that they perceived all faces as threatening to some extent, consistent with their enhanced fear vigilance during behavioral testing.

The aberrant dmPFC response and amygdala-dmPFC coupling during emotional face processing in the high-risk *v.* low-risk twins is consistent with a role of this circuitry in depression-linked pharmacology and maintenance of worry (Di *et al.* 2012; Robinson *et al.* 2013; Vytal *et al.* 2014). However, the stronger *negative* amygdala-dmPFC connectivity in high-risk twins contrasts with the greater *positive* amygdala-dmPFC coupling in response to negative stimuli under serotonin depletion or anticipatory anxiety (Robinson *et al.* 2013; Vytal *et al.* 2014). Negative amygdala-subgenual ACC coupling has been demonstrated during task-*irrelevant* processing of fearful faces (Pezawas *et al.* 2005), whereas positive coupling between the amygdala and the caudal part of dorsal ACC and dmPFC was associated with enhanced task-*relevant* threat processing (Robinson *et al.* 2012). Accordingly, we found that greater fear vigilance was associated with more negative amygdala-pregenual ACC coupling during (task-*irrelevant*) emotional face processing across all participants. This negative amygdala-pregenual ACC coupling may thus serve as a top-down mechanism inhibiting task-irrelevant threat processing. If so, the more negative coupling between amygdala and pregenual ACC-dmPFC in high-risk *v.* low-risk twins could indicate an acquired regulation strategy to compensate for their heightened subliminal fear vigilance, which interfered with task-relevant allocation of attentional resources. In keeping with this interpretation, high-risk twins showed impaired gender discrimination performance during face processing and this was correlated with their amygdala responsiveness to happy and fearful faces. However, gender discrimination accuracy showed no correlation with neural response to emotional faces in prefrontal or occipito-parietal regions. It is therefore unlikely that the enhanced prefrontal and occipito-parietal response in high-risk twins reflects lower task accuracy in this group.

Increased top-down inhibition of negative emotional processing could prevent onset of depression in this

https://doi.org/10.1017/S0033291714002542 Published online by Cambridge University Press

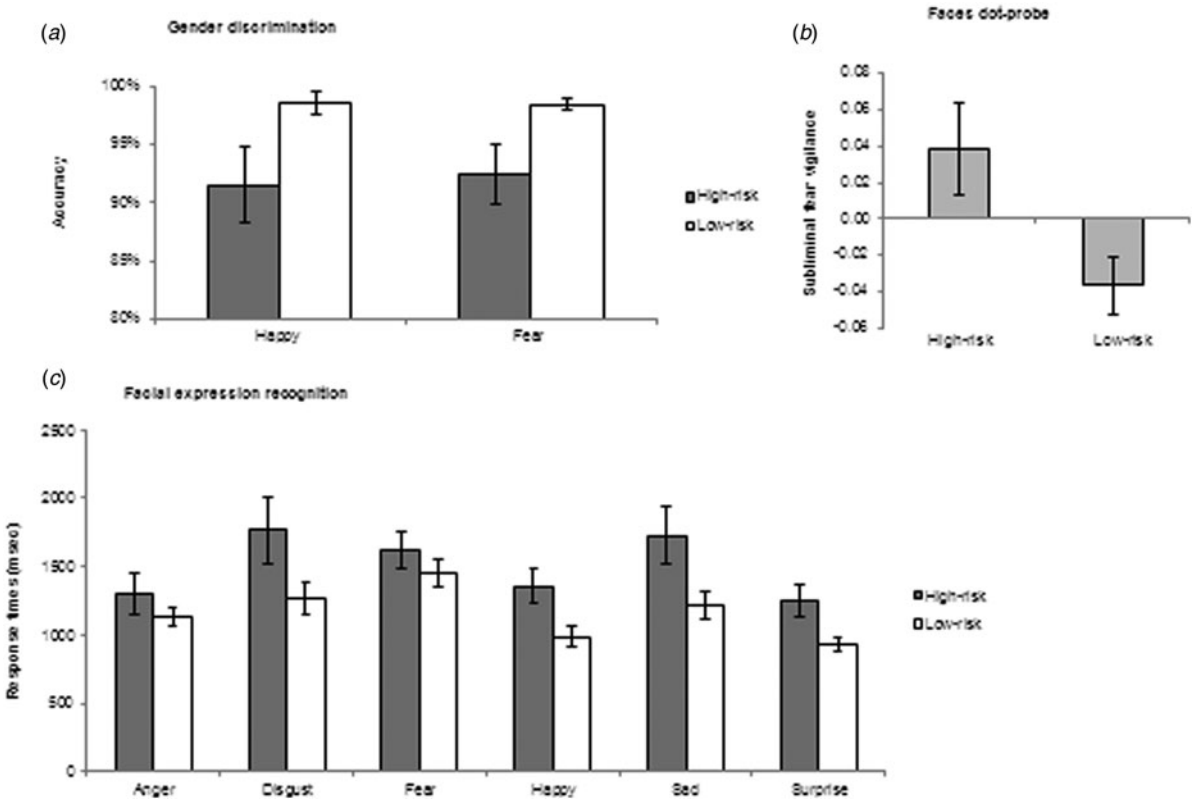

Fig. 3. Behavioural data. (*a*) Gender discrimination accuracy during emotional face processing in the scanner. High-risk twins showed reduced accuracy for both happy and fearful faces relative to controls. (*b*) Fear vigilance in the faces-dot probe test. High-risk twins displayed increased subliminal vigilance to fearful *v.* neutral faces, as reflected by greater accuracy for dots replacing masked fearful *v.* neutral faces. (*c*) Response times for correctly recognized emotional facial expressions. High-risk twins showed general increase in response latency for all emotional expressions compared to controls.

vulnerable population. Indeed, such compensatory cortical mechanisms are thought to be pivotal for clinical improvement with antidepressant drug therapy (Ruhe *et al.* 2012) and for prevention of relapse in recovered patients (Norbury *et al.* 2010; Goulden *et al.* 2012; Kerestes *et al.* 2012). Nevertheless, this increased effort to inhibit the interfering emotional reactivity may also explain high-risk twins' impairments in task performance during emotional face processing and facial expression recognition. Such effortful compensation strategies for emotional vulnerability may thus contribute to cognitive overload and potential development of depression in the face of stressful life events in this at-risk population.

The small sample size (with $N = 13$ in the at-risk group) was a limitation of the study, which reflects the scarcity of MZ twins in this follow-up study of the original larger sample (Vinberg *et al.* 2013). Nevertheless, we and others have demonstrated that inclusion of $N = 12$ participants per group matched for age and gender had a power of >0.8 to detect differences between groups in BOLD fMRI and cognitive response to emotional information (e.g. Miskowiak *et al.* 2007*a*, *b*). Another limitation was that participants' age was relatively high [mean age (S.D.): 45 (13) years], which was a consequence of the study being part of a 7-year follow-up assessment. Compensatory cortical control of emotional reactivity may therefore not be present in a younger cohort as indicated by Mannie *et al.* (2011). Consequently, the increased compensatory cortical control in our older sample may represent marker of resilience, as defined by effective coping and adaptation in the event of loss, hardship or adversity. However, several considerations speak against this. First, assessments of mood, personality and coping styles showed no greater resilience in our high-risk *v.* low-risk twins. Second, we have previously shown that these high-risk twins continue to be at equally high risk of developing depression also during middle age (Vinberg *et al.* 2013). Finally, the pattern of changes in neural and cognitive processing of emotional face stimuli was remarkably similar to findings in other at-risk populations (Goulden *et al.* 2012; Lisiecka *et al.* 2013; van Oostrom *et al.* 2013) and recovered depressed patients (Bouhuys *et al.* 1999; Bhagwagar *et al.* 2004; Leppanen *et al.* 2004; Anand *et al.* 2005*a*; Chen *et al.*

https://doi.org/10.1017/S0033291714002542 Published online by Cambridge University Press

2008; Dannlowski *et al.* 2009; LeMoult *et al.* 2009; Norbury *et al.* 2010; Erk *et al.* 2010; Kong *et al.* 2013). Nevertheless, additional investigation of younger MZ twins at high *v.* low risk for depression is warranted to better disentangle vulnerability and resilience mechanisms. On the other hand, it could also be argued that the relatively high age of our sample is a strength; by this age, one would expect any depression in the untested co-twins to have become apparent so we can be reasonably confident that the low-risk group here are truly at low risk. Had participants been 20 years younger, we would have been less confident about the low-risk group due to the possibility that depression in their co-twins may not yet have manifest itself. It is also important to further examine whether these at-risk individuals are indeed characterized by negative affective bias as we suggest here, or in contrast, by more general salience related differences in face processing. A strength of the study was the use of the unique Danish registers which enabled us to recruit MZ twins at high or low risk for depression in a study of neurocognitive endophenotype for depression. Further, the thorough longitudinal assessments of participants with psychiatric interviews and questionnaires over several years prior to this study enabled inclusion of only healthy, never-depressed twins at high *v.* low risk for depression, and avoided confounding factors such as current or past depressive episodes or medication which have well-documented effects on neural response to emotional information.

In conclusion, the study shows for the first time that MZ twins at high-risk for developing depression are characterized by abnormal neural response and functional connectivity within frontal, occipito-parietal and limbic regions during emotional face processing along with enhanced vigilance towards fearful faces. These findings add to the growing evidence for abnormalities in the processing of emotional faces as a key endophenotype for depression.

Acknowledgements

The study was funded by the Danish Council for Independent Research and Lundbeck Foundation. K.W.M.'s salary is partially funded by the Lundbeck Foundation. The authors thank the study participants for their time and effort.

Declaration of Interest

K.W.M. has received consultancy fees from Lundbeck. M.V. has been a consultant for Eli Lilly, Lundbeck, Servier and AstraZeneca. C.J.H. has received consultancy fees from P1vital Ltd, Servier, Eli Lilly, is a company director of Oxford Psychologists Ltd and has also received grant income from GSK, Lundbeck, Servier and AstraZeneca. H.R.S. has within the past 3 years received honoraria as reviewing editor for *Neuroimage*, as a speaker for Biogen Idec Denmark A/S, and scientific advisor for Lundbeck. L.V.K. has within the last 3 years been a consultant for Lundbeck, AstraZeneca and Servier. All other authors report no biomedical financial interests or potential conflicts of interest.

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