Psychiatry Research: Neuroimaging 291 (2019) 18–25

Contents lists available at ScienceDirect

Psychiatry Research: Neuroimaging

journal homepage: www.elsevier.com/locate/psychresns

A lack of differentiation in amygdala responses to fearful expression intensity in panic disorder patients

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ARTICLE INFO

Keywords:
Neuroimaging
Prefrontal cortex
Emotions
Facial expression
Anxiety

ABSTRACT

Patients with panic disorder show abnormalities in threat processing and regulation, both on a behavioural and neural level. Better understanding of the underlying mechanisms could help to develop new treatment strategies. In this study, we investigated brain region activation in 18 patients with untreated panic disorder (PD) and 17 healthy controls (HC) during the processing of emotional faces with fearful, happy and neutral expressions, using functional MRI. The intensity of the expressions was either prototypically high, medium or low. PD patients showed significantly increased activity in the dorso-medial prefrontal cortex (dmPFC) in response to faces in general and specifically for happy faces. While HC showed a decreased amygdala response to medium/low fearful versus high fearful faces, this effect was not present in PD: amygdala activation was stable across all fearful faces in this group. Psycho-physiological interaction analyses indicated more negative connectivity between the amygdala and prefrontal areas in the PD group during the task. Amygdala activation in panic patients appears to be less sensitive to decreasing intensities of fearful facial expressions and salience monitoring areas were less active during fearful faces in general in this group. This suggests PD patients might avoid more extensive processing of fearful faces.

1. Introduction

Panic disorder is amongst the most frequent anxiety disorders (Kessler et al., 2009), a class of disorders marked by a vast impact on life quality (Mendlowicz and Stein, 2000; Pollack and Marzol, 2000; Zaider et al., 2010) and a high economic burden (Greenberg et al., 1999; Koopmans et al., 2005; Leon et al., 1995; Weich and Lewis, 1998). Effective treatments such as cognitive behavioural therapy are available but are long and cost-intensive (Otto et al., 2000). Moreover, a subgroup of patients does not show clinically significant changes (Hans and Hiller, 2013). To improve interventions, it is essential to identify the underlying mechanisms of anxiety disorders, as these could represent key targets for treatment and serve as markers for treatment efficacy (Reinecke and Harmer, 2015).

Cognitive theories of anxiety underline the role of a selective bias towards threat-related information in cognitive processes such as attention and memory in the maintenance of the anxiety (Beck and Clark, 1997; Mathews and Mackintosh, 1998; Williams et al., 1997). Early automatic stages of processing are marked by hyper-vigilance towards threat, while subsequent deeper, more conscious processing is avoided (Amir et al., 1998; Bar-Haim et al., 2007; Cisler and Koster, 2010; Fox et al., 2002; Mogg and Bradley, 1998). Indeed, research has shown that patients with anxiety disorders present biases towards threat in automatic attention (Ashwin et al., 2012; Lange et al., 2011; Reinecke et al., 2011) and that for PD a reduction in this bias precedes and predicts a positive clinical outcome after treatment (Reinecke et al., 2013). The bias towards threat in patients with anxiety disorders is reflected in increased activation in the amygdala and other limbic areas (Ball et al., 2012; Etkin and Wager, 2007; Larson et al., 2006; Monk et al., 2008; Stein et al., 2002; van den Heuvel et al., 2005) implicated in the detection of threat (Ochsner et al., 2012; Whalen et al., 1998a). Moreover, a recent study on emotion regulation in PD patients indicates increased amygdala activity during viewing of aversive pictures, and that this hyperreactivity was reduced when PD patients applied specific cognitive strategies during an emotion regulation condition (Reinecke et al., 2015).

A similar amygdala activation pattern in individuals with panic disorder would be expected for threatening information from facial expressions. These stimuli are frequently used in tasks investigating emotion processing, as they are an ecologically valid way of conveying

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https://doi.org/10.1016/j.pscychresns.2019.07.002
Received 13 May 2019; Received in revised form 1 July 2019; Accepted 8 July 2019
Available online 09 July 2019
0925-4927/

emotions (Ekman, 1993) and have been frequently reported to elicit amygdala activity (Adolphs, 2002; Morris et al., 1998), even when they are subliminally presented (Morris et al., 2001; Whalen et al., 1998b). Remarkably, studies on emotional face processing in patients with panic disorder report the opposite – a hypoactivation of the amygdala in response to fearful faces - independent of the specific task that was used (Demenescu et al., 2013; Ottaviani et al., 2012; Pillay et al., 2006). However, it remains difficult to draw any strong conclusion about a specific reaction to emotional faces in panic disorder, as studies on this topic are scarce, with a small sample size, and typically included medicated patients. Medication can itself reduce amygdala responses (Chen et al., 2008; Pesold and Treit, 1995; Sheline et al., 2001) and therefore this is a substantial confound for the interpretation of these results. Moreover, the mentioned studies did not vary the intensity of the emotional expressions used in the experiments. Previous research in depression suggests that biases in emotional face processing can be identified best by measuring responses to emotional faces with increasing intensities (Chan et al., 2009; Fu et al., 2004; Surguladze et al., 2005).

In this study, we aim to investigate the specific pattern of brain region activation in response to emotional faces in unmedicated patients with panic disorder, using functional MRI and a gender identification task with emotional expressions. We hypothesize that activation in the amygdala in response to fearful faces will be increased in an unmedicated PD group compared to healthy controls, in line with most evidence on amygdala functioning in anxiety disorders. Moreover, to obtain a more fine-grained view of differences in emotion processing, emotional expressions with different intensities are used. We expect that PD patients show a steeper increase in amygdala activation as a function of fearful expression intensity compared to HC.

2. Methods

2.1. Participants

18 patients with panic disorder (PD; 10 with/ 8 without agoraphobia) and 17 healthy controls (HC)[1] without axis-I history were recruited from the general public based on their scores on the Panic Disorder Severity Scale (PDSS) (Houck et al., 2002), which had to be 0 in the HC group and at least 4 in the PD group (9.3 ± 5.7). Diagnoses were assessed using the Structured Clinical Interview for DSM-IV Axis I Disorders (First et al., 1996). Three PD fulfilled criteria for comorbid specific phobia, with panic disorder being the primary diagnosis. General exclusion criteria were left-handedness, MRI contraindications, epilepsy, current or past psychotic disorder, bipolar disorder, or substance abuse, and antidepressant or psychological treatment during the last 6 months. Groups were matched with regards to gender, age, years of education and verbal intelligence as measured with the National Adult Reading Test (Nelson, 1982) (Table 1). Three PD having reported on-demand benzodiazepine or propranolol intake were medication free 48 h before scanning. Ethical approval was obtained from the local research ethics committee.

2.2. Clinical symptoms and subjective state

At baseline, participants completed the Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983), Body Sensations Questionnaire BSQ and Agoraphobic Cognitions Questionnaire (ACQ) (Chambless et al., 1984). Before and after the scan, they also completed the Positive and Negative Affect Schedule (PANAS) (Watson et al., 1988) and the state scale of the State Trait Anxiety Inventory (STAIS) (Spielberger et al., 1983). Clinical symptom and subjective state data were analysed running independent-samples t-tests in SPSS 19 (IBM Corp., USA).

[1] Outliers were removed based on Tabachnik and Fidell (2001), which led to the exclusion of one healthy volunteer with amygdala signal during medium fearful faces above 3 standard deviations of the total mean.

Table 1
Means (+/− SD) for socio-demographic and questionnaire data, state measures and behavioural data for both groups.

	PD (n = 18)	HC (n = 17)	p*
Socio-demographic and questionnaire data			
Gender	14/4	14/3	0.735
Age	36.5 (13.8)	34.1 (11.7)	0.587
Years of education	16.6 (2.7)	17.9 (4.2)	0.299
NART	117.8 (5.1)	118.5 (3.9)	0.648
HADS Anxiety	14.6 (4.062)	2.2 (1.954)	<0.01
HADS Depression	8.1 (3.376)	0.7 (0.996)	<0.01
ACQ	2.4 (0.6)	1.1 (0.3)	<0.01
BSQ	3.4 (0.7)	1.3 (0.3)	<0.01
State measures			
Pre-scan:			
PANAS positive	26.9 (10.3)	35.9 (5.6)	<0.01
PANAS negative	22.6 (5.1)	10.5 (1.1)	<0.01
STAIS	50.2 (11.9)	24.1 (4.2)	<0.01
Post-scan:			
PANAS positive	28.1 (11.3)	33.1 (5.7)	0.109
PANAS negative	21.8 (6.6)	12.6 (5.5)	<0.01
STAIS	41.9 (12.1)	27.3 (8.8)	<0.01
Behavioural measures			
Accuracy:			
Fearful faces			
High	91.2 (8.9)	89.7 (8.6)	0.62
Medium	94.7 (4.9)	93.6 (6.1)	0.58
Low	93.3 (6.4)	95.1 (7.5)	0.45
Happy faces			
High	94 (5.9)	93.1 (7.2)	0.71
Medium	95.6 (6.8)	95.6 (5.0)	0.99
Low	94 (6.1)	94.9 (5.6)	0.66
Neutral faces	93.1 (5.2)	94.6 (5.1)	0.38
RT:			
Fearful faces			
High	709.5 (191.3)	762.4 (181.6)	0.41
Medium	718.9 (215.2)	749.5 (187.3)	0.66
Low	697.5 (215.5)	748.1 (196.1)	0.47
Happy faces			
High	713.7 (213)	741.6 (177.4)	0.68
Medium	707.2 (191.6)	755.5 (167.6)	0.43
Low	683 (176.3)	746.9 (162.6)	0.27
Neutral faces	698.3 (195.2)	746.3 (175.2)	0.45

*p-values from independent sample t-tests, except for the p-value of gender, which results from a Chi-square test.

2.3. fMRI task design

All subjects participated in a 16-min gender identification task using rapid event-related fMRI (Chan et al., 2009). Stimuli were photographs of 8 faces (4 male, 4 female) with fearful, happy or neutral expressions (Ekman and Friesen, 1975; Young et al., 1997). The intensity of the fearful and happy facial expressions was either prototypical/high (100%), medium (60%) or low (30%) as a result of a morphing procedure (see Chan et al., 2009) yielding 7 different facial expressions and 56 stimuli in total. 192 trials (3 presentations of each stimulus and 24 presentations of a fixation cross as baseline) were presented on an opaque screen in random order for 500 ms using E-prime version 1.0 (Psychology Software Tools Inc., USA). The inter-trial interval varied according to a Poisson distribution with a mean of 5000 ms. Participants were instructed to indicate the gender of each face using a MRI-compatible button-box. Accuracy and reaction times were recorded using E-Prime.

2.4. Image acquisition

Images were obtained using a 3T Siemens Sonata scanner. T_2*-

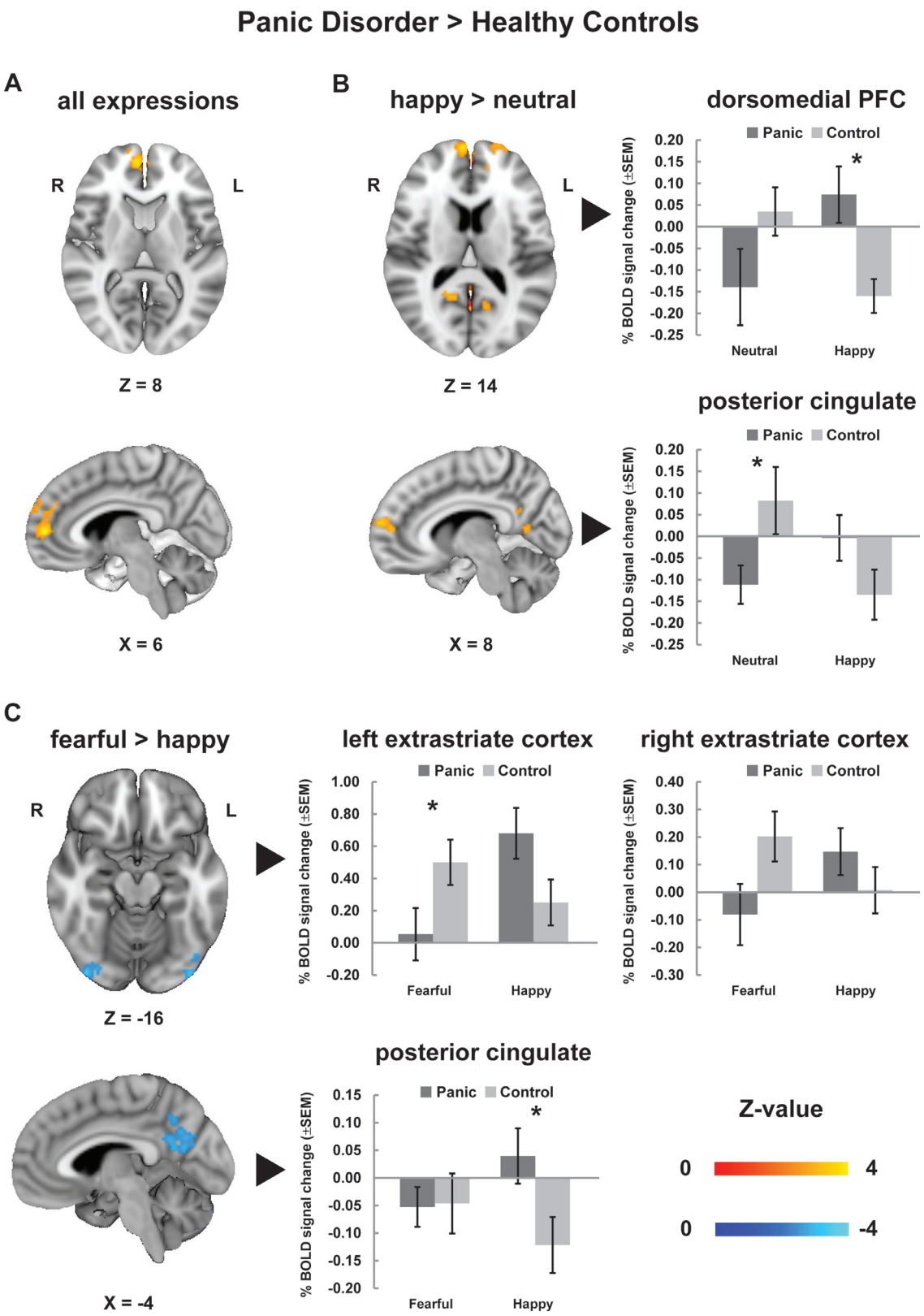

Fig. 1. Clusters from the whole brain analysis (A) For faces in general (independent of emotional valence) the PD group showed more activation in the dmPFC cortex. **(B)** In response to happy versus neutral faces, the PD group showed increased activation in the dorsomedial prefrontal cortex and posterior cingulate. The dorsomedial PFC showed increased activation in response to happy faces in PD compared to HC. In the posterior cingulate, PD showed a decreased response to neutral faces compared to HC. **(C)** In response to fearful > happy faces, the control group showed increased activation in the left and right extrastriate cortex and posterior cingulate compared to the PD group. In the left extrastriate cortex, PD showed decreased activation in response to fearful faces compared to HC. In the posterior cingulate, there was increased activation in PD compared to HC in response to happy faces. (* = $p < 0.05$; Letters below brain slices indicate corresponding MNI-coordinates; colour-bar indicates *Z*-value and applies to all shown activation clusters).

Table 2
Clusters from whole brain analysis contrasts of interest.

	BA	Side	Cluster size (voxels)	MNI (*x*, *y*, *z*)	*Z*-score
Main effect of group (PD > HC)					
Dorsal PFC	10	R/L	453	6, 54, 6	−3.85
Happy versus neutral (PD > HC)					
Medial PFC	10	R/L	472	6, 62, 14	3.38
Posterior cingulate cortex	23/30	R/L			
Fearful versus neutral (PD > HC)					
Extrastriate cortex	19	R	306	38, −86, −18	3.23
	19	L	354	−40, −86, −20	3.47
Ventral posterior cingulate/precuneus	23/30	R/L	413	−6, −56, 18	3.04

BA = Brodmann area, PD = panic disorder, HC = healthy control, PFC = prefrontal cortex, R = right, L = left.

weighted functional data were acquired for a whole-brain field-of-view (64 × 64 × 40 matrix, voxel resolution 3 mm^3, repetition time = 3000 ms, echo time = 30 ms, flip angle = 90°). Field maps were acquired using a dual echo 2D gradient echo sequence with echoes at 5.19 and 7.65 ms, and a repetition time of 500 ms. High-resolution T_1-weighted images were acquired for subject alignment, using an MPRAGE sequence (174 × 192 × 192 matrix, voxel resolution 1 mm^3, repetition time = 2040 ms, echo time = 4.7 ms, inversion time = 900 ms).

2.5. Image analysis

Imaging data were analysed using FSL (FMRIB Software Library; www.fmrib.ox.ac.ul/fsl). Pre-processing included motion correction (Jenkinson et al., 2002), non-brain removal (Smith, 2002), spatial smoothing (Gaussian kernel FWHM = 5.0 mm), grand-mean intensity normalisation of the entire 4D dataset by a single multiplicative factor, registration of the functional space template to the anatomical space and the MNI 152 space, high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma = 50.0 s) and fieldmap correction. At the first-level, data were analyzed using a general linear model approach with local autocorrelation correction (Woolrich et al., 2001). Seven regressors of interest were included: intensity (low, medium, high) of fear and happy as well as neutral. The main contrasts of interest were i) faces versus baseline in general, ii) fear versus happy expressions (and vice versa) for each intensity level, and iii) fearful vs. neutral, happy vs. neutral, and fearful vs. happy (and vice-versa) faces collapsed across the different intensity levels. These individual activation maps were then entered into the group level (PD, HC), using a mixed-effects analysis across the whole brain (Beckmann et al., 2003). *Z*-statistic functional MRI images were thresholded using clusters determined by $Z > 2.3$ and a (corrected) cluster significance threshold of $p = .05$.

Due to strong evidence implicating the amygdala in threat processing (Reinecke and Harmer, 2015), region of interest analyses (ROI) were carried out for the anatomical left and right amygdala (Oxford-Harvard atlas in FSL; thresholded at 0.5). Significant whole-brain or ROI interactions were explored by extracting BOLD signal changes within these areas and entering these into Group x Emotion mixed-design ANOVAs and appropriate follow-up t-tests.

A psychophysiological interaction (PPI) analysis was conducted to identify brain regions in which activity covaried with the left or right amygdala in one or both groups and one or multiple conditions (i.e. 7 expression intensities). For each participant, we extracted the deconvolved time series from the left and right amygdala (defined by the Harvard-Oxford anatomical atlas), reflecting amygdala activity during picture blocks versus baseline. The time series were entered into two separate PPI analyses – one for each amygdala seed region, along with the psychological regressors (7 expression intensities) and the seven PPI regressors (expression × time series for each intensity level). The contrast images resulting from these individual PPI analyses (first-level) were entered into a mixed-effects group analysis across the whole brain.

3. Results

3.1. Affective ratings and behavioural data

Compared to controls, PD patients showed significantly higher anxiety and depression (HADS), fear of physical symptoms (BSQ) and agoraphobic cognitions (ACQ) (all $p < 0.01$). PD reported higher negative affect and anxiety (PANAS negative, STAIS) as well as lower positive affect (PANAS positive) before the scan (all $p < 0.05$). After the scan, the differences in negative affect and anxiety persisted, but positive affect ratings were similar for both groups. Regarding the behavioural data, there were no significant differences between the groups in overall gender discrimination accuracy and mean reaction time. Moreover, no group differences were detected when analyzing the reaction times and accuracy for each emotional condition separately (all $p > 0.2$) (Table 1).

3.2. BOLD functional MRI: whole-brain analysis

Compared to HC, PD showed significantly increased activity in the dorso-medial PFC in response to faces in general (versus baseline), independent of emotion (Fig. 1A and Table 2). There were no significant group differences for fearful faces contrasted against neutral faces.

In response to happy versus neutral faces, collapsed across all intensity levels, we found increased activation in the dorso-medial PFC and posterior cingulate cortex for the PD group compared to the HC group (Fig. 1B and Table 2). Post-hoc analyses on BOLD signal change extracted from the two clusters showed that the interaction in the frontal cluster was driven by a group difference in the activation in response to happy faces, with PD showing higher activation than HC. The interaction in the posterior cingulate cluster was based on PD showing a decreased response to neutral faces compared to HC (Table 3).

Table 3
Test values follow-up analyses whole-brain interaction effects and region of interest analyses

	Test value	*p*-value
Whole brain analyses (follow-up interactions)		
Dorsomedial PFC [happy versus neutral]		
Happy (PD > HC)	$t(33) = 3.04$	.005
Neutral (PD > HC)	$t(33) = -1.65$	.109
Posterior cingulate [happy versus neutral]		
Happy (PD > HC)	$t(33) = 1.68$	.103
Neutral (PD > HC)	$t(33) = -2.20$	.035
Posterior cingulate [fearful versus happy]		
Happy (PD > HC)	$t(33) = 2.26$	.030
Fearful (PD > HC)	$t(33) = -0.10$	.923
Left extrastriate cortex [fearful versus happy]		
Happy (PD > HC)	$t(33) = 2.01$	.053
Fearful (PD > HC)	$t(33) = -2.08$	.046
Right extrastriate cortex [fearful versus happy]		
Happy (PD > HC)	$t(33) = 1.17$	.250
Fearful (PD > HC)	$t(33) = -1.96$	.059
Region of interest analyses: Amydala		
Group × Intensity	$F_{2,66} = 3.38$	.040
PD: Intensity(high – medium – low fearful)	$F_{2,34} = 0.36$	.698
HC: Intensity(high – medium – low fearful)	$F_{2,32} = 4.83$	.015
HC: high fearful vs. medium fearful (post-hoc)		.026
HC: high fearful vs. low fearful (post-hoc)		.014
HC: medium fearful vs low fearful (post-hoc)		.579

PD = panic disorder, HC = healthy control, PFC = prefrontal cortex.

In response to fearful versus happy faces, collapsed across all intensity levels, we found decreased activation in the left and right extrastriate cortex and in the ventral posterior cingulate/precuneus cortex for the PD group compared to HC group (Fig. 1C and Table 2). Post-hoc analyses on BOLD signal change extracted from each of these clusters indicated that the *precuneus* group x emotion interaction was driven by a group difference in response to happy faces, with PD showing significantly higher activation than HC. The interaction in the *left extrastriate cluster* was based on PD showing a decrease in activation in this area in response to fearful faces and a non-significant trend towards higher activation in response to happy faces compared to HC. The interaction in the *right extrastriate cluster* showed a similar pattern, but was not driven by significant group differences within specific conditions. For PD, there was a decrease in activation in this cluster for fearful compared to happy faces, while there was an increase in activation in HC (Table 3).

3.3. BOLD functional MRI: amygdala ROI analyses

A Hemisphere x Group x Emotion (all fearful faces vs. all happy faces) ANOVA for the BOLD signal extraction from the left and right amygdala revealed no laterality differences, group differences or group - emotion interactions (all $F < 2.8$, all $p > 0.05$). A Hemisphere x Group x Intensity level (low fearful – medium fearful – high fearful) ANOVA for fearful faces revealed a significant Group x Intensity interaction, without any laterality differences (all $F < 1.86$, all $p > 0.05$). A follow-up Intensity level ANOVA for each group showed that intensity had an effect on amygdala activation for HC, but not for PD. Post-hoc pairwise comparisons for HC revealed that medium fearful faces and low fearful faces elicited lower amygdala activity than high fearful faces (Fig. 2 and Table 3). In summary, amygdala activation was decreased for medium and low fearful faces compared to high fearful faces in control participants, whereas activation was stable across all fearful faces in the patient group.

3.4. BOLD functional MRI: PPI analyses

During the whole task, there was less positive connectivity between the bilateral amygdala (seed region) and widespread clusters in the PFC, including bilateral dlPFC and dmPFC, for PD compared to HC. Moreover, patients showed less positive connectivity between the left amygdala and the bilateral anterior insula (See Fig. 3).

4. Discussion

This study indicates that untreated patients with panic disorder show a different pattern of brain activation in response to emotional faces compared to healthy controls. Dorso-medial prefrontal cortex (dmPFC) activation during the presentation of emotional faces in general was higher in the PD group. For happy compared to neutral faces, we found increased dmPFC and posterior cingulate activation in the PD group. For fearful relative to happy faces, PD patients showed less extra-striate and posterior cingulate activation. Within the fearful faces condition, we found an effect of emotion intensity on amygdala activation in the healthy control group, with low-intensity fearful faces provoking lower amygdala activation than high-intensity faces. In contrast, amygdala activation was not affected by the intensity of the fearful expressions in the PD group. The PD group showed more pronounced negative connectivity between the amygdala and prefrontal areas in the PD group during the presentation of faces in general.

Our hypothesis that PD patients would show increased amygdala activation in response to fearful faces was not confirmed. Instead, we found evidence for a reduced sensitivity of the amygdala for differences in the intensity of fearful facial expressions in panic disorder. Neurobiological theories of panic disorders address an important role to the amygdala, which is thought to be hypersensitive in patients (Gorman et al., 2000). For example, panic patients show higher amygdala activation when viewing affective pictures (Reinecke et al., 2015) and in response to panic-related words in a colour-naming task (van den Heuvel et al., 2005). Moreover, case reports of patients experiencing a panic attack in the scanner also point at the involvement of the amygdala (Dresler et al., 2011; Pfleiderer et al., 2007). The structure is highly connected to regions responsible for the bodily expression of fear, such as the peri-aqueductal grey (PAG) and the hypothalamus (Rodrigues et al., 2009).

However, there are also studies reporting amygdala hypo-activation, especially in the context of fearful expression processing. Using a free viewing task, Pillay et al. (2006) found reduced activation in PD response to fearful faces. Moreover, in contrast to healthy participants, PD patients did not show increased amygdala activation in response to masked fearful faces (Ottaviani et al., 2012). Demenescu et al. (2013) also reported reduced amygdala activation in panic patients in response to facial expressions in general (i.e. angry, fearful, happy and neutral), using a similar gender identification task. In sum, these studies suggest that amygdala activity in panic disorder, compared to healthy controls, seems to be less sensitive to the presentation of fearful expressions per se, regardless of whether the faces are presented explicitly, implicitly or subluminally. This blunted amygdala response has been suggested to be due to chronic hyperarousal (Ottaviani et al., 2012; Pillay et al., 2006).

In the current study with unmedicated patients and a larger sample size, we did not find evidence for hypo- or hyperactivation of the amygdala in panic disorder in response to fearful faces in general. Instead, we found that amygdala activation was dependent on fearful face *intensity* in healthy controls, while there was a lack of differentiation in amygdala activity in panic disorder patients. An explanation for this might be that patients, even though they are able to identify the

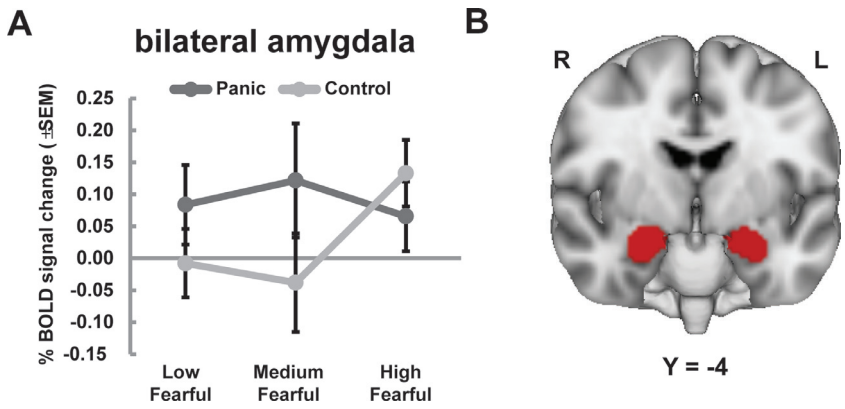

Fig. 2. **(A)** Within the fear intensity conditions, amygdala response was stable for PD, but decreased in HC for low and medium fearful faces compared to high fearful faces. **(B)** Regions of interest: the left and right amygdala. Masks are based on the Oxford-Harvard anatomical atlas using FSL (Jenkinson et al., 2012).

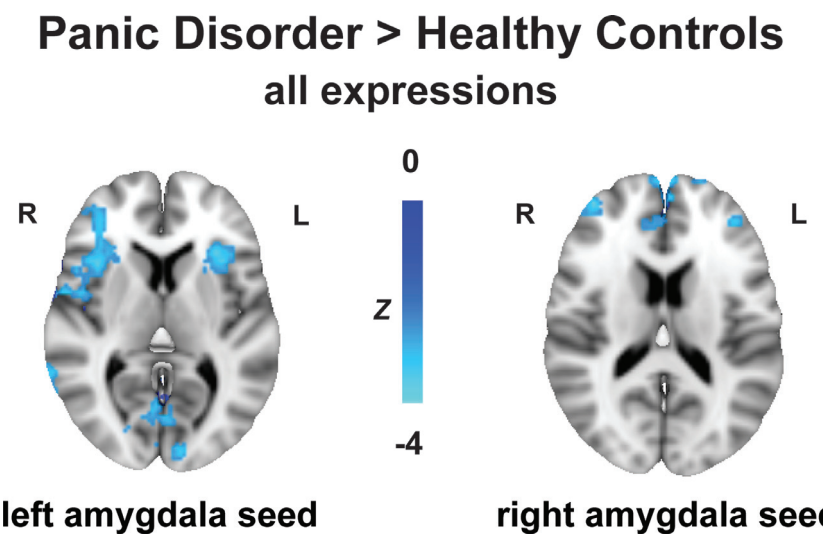

Fig. 3. Functional connectivity analyses. Compared to controls, patients showed more negative connectivity between the bilateral amygdala and multiple regions including clusters in the PFC, anterior insula and precuneus/posterior cingulate during the whole task.

gender of the faces, tend to avoid more extensive processing of the expressions during this specific task, and thereby process the fearful faces to a lesser extent. Avoidance is an important characteristic of anxiety disorders (Hofmann et al., 2012; Mogg et al., 2004). In accordance with this explanation, we found lower extrastriate cortex activation in the PD group during the presentation of fearful vs happy faces. This may indicate reduced visual processing of fearful faces, especially because this activation cluster overlaps with one of the clusters described in a meta-analysis of emotional face processing tasks (Sabatinelli et al., 2011). Moreover, using eye-tracking, a bias away from the emotionally salient eye regions has been shown in highly neurotic individuals and social phobics (Di Simplicio et al., 2014; Horley et al., 2003), indicating that avoidance of emotional expressions indeed plays a role in anxious individuals.

The pattern of dorsomedial prefrontal cortex (dmPFC) and posterior cingulate cortex (PCC) activation in panic disorder patients seems to be in line with a tendency to avoid fearful face processing in this group. Our patients showed more activation of the dmPFC compared to controls for faces in general, and specifically for happy compared to neutral faces. This is complemented by the functional connectivity analysis, which showed increased negative coupling in patients with panic disorder between the amygdala and various prefrontal areas and the insula for emotional faces in general. The dmPFC has been implicated in monitoring emotional conflict (Etkin et al., 2006) and emotional awareness (Etkin and Wager, 2007; Taylor et al., 2003). Our finding of increased activation in panic disorder in this area for happy faces and reduced connectivity with the amygdala for fearful faces seems surprising: one would expect fearful faces to be more salient than happy faces in this group and *increased* dmPFC-amygdala coupling has been shown before for threatening scenes in PD (Reinecke et al., 2015). However, it fits with the reduced visual cortex activation for fearful vs. happy faces in panic disorder we found here and our interpretation that processing of fearful faces is avoided in this patient group.

In the posterior cingulate cortex, we found more activation in the panic disorder group for happy faces compared to both neutral and fearful faces. Cingulate activation in response to happy faces in PD has been reported before (Pillay et al., 2007), though in the more anterior part, which is implicated in conflict/performance monitoring (Botvinick et al., 1999; MacDonald, 2000). Panic disorder-related effects in specifically the posterior region of the cingulate cortex have been found during reacting to panic-related words (Maddock et al., 2003) and during resting state fMRI (Pannekoek et al., 2013). The PCC has been implicated in the integration of valence and spatial attention, which is crucial for directing attention towards motivationally relevant stimuli (Leech and Sharp, 2014; Mohanty et al., 2008). In the current study, patients might have seen happy faces as safety signal, and therefore directed their attention towards them. Evidence for a focus on positive expressions in PD was found before in a study showing that patients are better at recognizing faces they had previously rated as 'safe' (Lundh et al., 1998).

Some limitations need to be taken into account. Our results suggest that group differences in attention towards emotional expressions might have played an important role. However, we did not assess attention directly. An interesting follow-up avenue would be to use eye-tracking during the fMRI task, to investigate whether reduced amygdala sensitivity for threatening facial expressions in PD could be attributed to avoidance of the emotionally salient eye region. Competition of multiple faces might be a prerequisite for hypervigilance towards threat to occur (Bar-Haim et al., 2007). Without this competition, panic patients might be able to counterbalance the hyper-reactivity of the fear network by avoiding more extensive processing the stimuli. Other paradigms, such as the dot-probe task with emotional faces (Monk et al., 2008), might be more suitable to tap into hypervigilance in this patient group.

The PD group had significantly higher levels of depression than the HC group. While this is a confound, it does not explain the pattern of increased activation in response to happy faces found in PD in this study, because depressed or at-risk individuals typically show decreased responses toward happy expressions (Chan et al., 2009; Surguladze et al., 2005).

Taken together, panic disorder patients did not show the expected differences in activation in the amygdala for fearful faces with different intensities, nor higher activation in brain regions implicated in salience monitoring in response to threat in general. Instead, they showed increased activation in attention and salience monitoring areas for happy faces. Possibly, patients were able to avoid thorough processing of fearful faces and were monitoring for positive expressions that could function as a safety signal, which might actually be two sides of the same coin.

Declaration of interest

Dr C.J. Harmer has served as a consultant for P1vital, GlaxoSmithKline, Servier, Astra Zeneca, Johnson & Johnson and Lundbeck and is on the advisory board and holds shares of P1vital. The remaining authors declare no conflict of interest.

Acknowledgements

Preparation of this paper was supported by a grant from the Medical Research Council to Catherine J. Harmer (G0501223). Andrea Reinecke is supported by a fellowship from MQ: Transforming Mental Health (MQ14F192). CJH is supported by the NIHR Oxford Health Biomedical Research Centre. *The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.*

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