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Effective emotion regulation strategies improve fMRI and ECG markers of psychopathology in panic disorder: implications for psychological treatment action

A Reinecke1, N Filippini1,2, C Berna1,2,3, DG Western4,5, B Hanson6, MJ Cooper6, P Taggart7 and CJ Harmer1

Impairments in emotion regulation are thought to have a key role in the pathogenesis of anxiety disorders, but the neurobiological underpinnings contributing to vulnerability remain poorly understood. It has been a long-held view that exaggerated fear is linked to hyperresponsivity of limbic brain areas and impaired recruitment of prefrontal control. However, increasing evidence suggests that prefrontal–cortical networks are hyperactive during threat processing in anxiety disorders. This study directly explored limbic–prefrontal neural response, connectivity and heart-rate variability (HRV) in patients with a severe anxiety disorder during incidental versus intentional emotion regulation. During 3 Tesla functional magnetic resonance imaging, 18 participants with panic disorder and 18 healthy controls performed an emotion regulation task. They either viewed negative images naturally (Maintain), or they were instructed to intentionally downregulate negative affect using previously taught strategies of cognitive reappraisal (Reappraisal). Electrocardiograms were recorded throughout to provide a functional measure of regulation and emotional processing. Compared with controls, patients showed increased neural activation in limbic–prefrontal areas and reduced HRV during incidental emotion regulation (Maintain). During intentional regulation (Reappraisal), group differences were significantly attenuated. These findings emphasize patients’ ability to regulate negative affect if provided with adaptive strategies. They also bring prefrontal hyperactivation forward as a potential mechanism of psychopathology in anxiety disorders. Although these results challenge models proposing impaired allocation of prefrontal resources as a key characteristic of anxiety disorders, they are in line with more recent neurobiological frameworks suggesting that prefrontal hyperactivation might reflect increased utilisation of maladaptive regulation strategies quintessential for anxiety disorders.

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INTRODUCTION

Anxiety disorders are very common and disabling conditions that cause a particularly high economic burden1–4 and the problem remains that not all patients show stable benefits in response to first-line intervention approaches.5,6 To improve treatments and their application, it is essential to define key mechanisms underlying anxiety disorders, as these are likely to represent important targets for treatment. Clinical models of anxiety propose that impairments in the regulation of negative affect have an important role in the pathogenesis of a disorder, as they contribute to exaggerated fear responses.7,8 Following neurobiological accounts of emotion regulation, the processing of threat involves signalling in limbic brain regions such as the amygdala, a key area implicated in the fast automatic registration of threat, whereas successful downregulation of this response is thought to be associated with increased recruitment of prefrontal areas of cognitive control.9,10 This is well supported by studies showing that in healthy volunteers, deliberate downregulation of negative affect is correlated with increased activation in medial and lateral areas of the prefrontal cortex (PFC), and that such activation dampens limbic signalling.11,12 In contrast, anxiety disorders are proposed to be associated with hyperresponsivity of limbic brain areas and impaired recruitment of prefrontal control.13,14

However, a number of novel findings suggest that while decreased allocation of lateral and ventral prefrontal resources seems to be an important characteristic of participants with non-clinical high trait anxiety or worry,15,16 activation in these areas is more likely to be increased in clinical anxiety disorders during threat processing. In particular, studies have reported increased activation in the dorsal anterior cingulate cortex (ACC) and dorsomedial PFC (dmPFC) in specific phobia,17 social anxiety disorder,18,19 panic disorder20,21 and generalised anxiety disorder.22 Similarly, anxiety-specific threat processing has increasingly been associated with heightened activation in dorsolateral (dPFC),23,24 ventrolateral (vPFC)20,21,25 and ventromedial PFC (vmPFC)23,26,27

Recent neurobiological accounts of anxiety disorders argue that, different from a view assuming reduced prefrontal cognitive control, prefrontal hyperactivation might reflect increased utilisation of dysfunctional regulation attempts in anxiety disorders.28,29 This is in line with anxiety disorders being associated with the development of avoidance and safety strategies, such as escaping the anxiety-provoking situation, or mental distraction from the threat stimulus. These avoidance-based strategies are believed to

1Department of Psychiatry, University of Oxford, Warneford Hospital, Oxford, UK; 2Centre for Functional Magnetic Resonance Imaging of the Brain, University of Oxford, Oxford, UK; 3Service d’anesthésiologie Centre Hospitalier, Université Vaudoise, Lausanne, Switzerland; 4Department of Mechanical Engineering, University College London, London, UK; 5Department of Mechanical Engineering, University of Bristol, Bristol, UK; 6Isis Education Centre, University of Oxford, Oxford, UK and 7Institute of Cardiovascular Sciences, University College London, London, UK. Correspondence: Dr A Reinecke, Department of Psychiatry, University of Oxford, Warneford Hospital, Oxford OX37JX, UK.

E-mail: andreareinecke@psych.ox.ac.uk
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Table 1. Socioeconomic, mood and anxiety self-report, negative affect ratings and heart-rate variability scores in the two groups (mean ± s.d., independent-samples t-test/*t-test P-scores)

|                          | Panic patients | Healthy controls | P-score |
|--------------------------|----------------|------------------|---------|
| **Sociodemographic data**|                |                  |         |
| Age (years)              | 36.3 ± 13.8    | 32.3 ± 12.1      | 0.341   |
| Gender                   | 14 female/     | 14 female/       | 0.655   |
|                          | 4 male         | 4 male           |         |
| Years of education       | 16.6 ± 2.7     | 17.5 ± 4.2       | 0.473   |
| Verbal IQ (NART)         | 117.8 ± 5.1    | 118.8 ± 3.9      | 0.526   |
| **Depression and trait anxiety** |            |                  |         |
| HADS—anxiety            | 14.6 ± 4.1     | 2.0 ± 1.6        | < 0.001 |
| HADS—depression         | 8.1 ± 3.4      | 0.7 ± 1.0        | < 0.001 |
| **Panic symptoms**       |                |                  |         |
| BSQ                      | 3.4 ± 0.7      | 1.4 ± 0.4        | < 0.001 |
| ACQ                      | 2.4 ± 0.6      | 1.4 ± 0.4        | < 0.001 |
| **Heart rate variability** |              |                  |         |
| N = 15                   |                |                  |         |
| LF/HF Maintain           | 2.8 ± 2.2      | 1.4 ± 1.0        | < 0.047 |
| LF/HF Reappraisal        | 2.0 ± 1.6      | 1.7 ± 1.1        | NS      |
| **Negative affect ratings** |            |                  |         |
| Maintain                 | 2.8 ± 0.6      | 2.9 ± 0.6        | NS      |
| Reappraisal              | 1.9 ± 0.6      | 1.9 ± 0.7        | NS      |
| **State mood pre MRI scan** |          |                  |         |
| Anxious                  | 67.7 ± 23.5    | 4.3 ± 6.1        | < 0.001 |
| Sad                      | 36.6 ± 28.9    | 7.7 ± 15.2       | 0.001   |
| Calm                     | 34.7 ± 23.5    | 85.0 ± 11.2      | < 0.001 |
| Happy                    | 49.3 ± 24.4    | 75.3 ± 12.8      | < 0.001 |
| **State mood post MRI scan** |           |                  |         |
| Anxious                  | 29.9 ± 28.1    | 9.0 ± 21.6       | 0.017   |
| Sad                      | 31.2 ± 22.7    | 6.1 ± 10.9       | < 0.001 |
| Calm                     | 54.3 ± 27.4    | 79.7 ± 18.7      | 0.003   |
| Happy                    | 53.1 ± 20.3    | 70.8 ± 17.3      | 0.008   |

Abbreviations: ACQ, Agoraphobic Cognitions Questionnaire; BSQ, Body Sensations Questionnaire; HADS, Hospital Anxiety and Depression Scale; IQ, intelligence quotient; LF/HF, low-frequency/high-frequency heart-rate variability ratio; MRI, magnetic resonance imaging; NART, National Adult Reading Test.

Responses to threat images during incidental emotion regulation where pictures are viewed naturally, and intentional emotion regulation where patients use previously learned strategies of cognitive reappraisal similar to those typically taught in CBT. Furthermore, we measured beat-to-beat heart-rate variability (HRV), a measure of autonomic innervation of the brain to the heart, as an additional indicator of emotion control.\textsuperscript{17,34} Increased HRV in response to stressful stimuli reflects a dominance of the parasympathetic over the sympathetic influence and therefore successful emotion regulation.\textsuperscript{29} In line with this, reduced HRV has been reported in patients with anxiety disorders.\textsuperscript{30,41} We hypothesised that during incidental emotion regulation, patients would draw on their maladaptive regulation strategies, reflected in increased PFC activation, ineffective downregulation of limbic activation and reduced HRV. In contrast, we expected these limbic–prefrontal activation patterns and HRV reductions to be dampened during intentional regulation, where alternative, adaptive control strategies would be used.

**MATERIALS AND METHODS**

**Participants**

Following a priori power calculations, 18 unmedicated patients with panic disorder (10 with/without agoraphobia) naive to psychological treatment and 18 healthy controls without Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)\textsuperscript{42} axis-I history were recruited for the public (Table 1). Statistical power information was derived from behavioural data gained in a previous study using a faces dot-probe task in patients versus healthy controls.\textsuperscript{43} These calculations suggested that with an alpha level of 5%, sample sizes of 18 per group would be sufficient to gain statistical power of 80% (condition masked fearful faces: patients M = 31, s.d. = 34, controls M = 27, s.d. = 35). Diagnoses were assessed using the Structured Clinical Interview for DSM-IV Axis I Disorders.\textsuperscript{44} Three patients fulfilled criteria for comorbid-specific phobia, with panic disorder being the primary diagnosis. General exclusion criteria were left-handedness, magnetic resonance imaging (MRI) contraindications, epilepsy, history of psychotic, bipolar or substance abuse disorder, and antidepressant treatment during the last 6 months. Three patients having reported occasional (but not regular) on-demand benzodiazepine or prazepam intake were medication-free 48 h before scanning. Ethical approval was obtained from the local research ethics committee.

**Mood and subjective state**

Participants completed the Hospital Anxiety and Depression Scale,\textsuperscript{46} Body Sensations Questionnaire and Agoraphobic Cognitions Questionnaire.\textsuperscript{47} Before and after the scan, they completed Visual Analogue Scales for the dimensions anxious, sad, calm and happy (0–100 mm, not at all—extremely) to assess state mood.

**fMRI task design**

Stimuli were 40 negatively valenced coloured IAPS images\textsuperscript{48} picturing characteristic panic-related catastrophic expectations, such as accidents or funerals (mean valence ratings 2.8 ± 1.7, mean arousal ratings 6.0 ± 2.2 on 9-point Likert scales ranging from 1 = unpleasant/low arousal to 9 = pleasant/high arousal). Valence and arousal ratings as well as scene content were matched between the two experimental conditions Maintain and Reappraisal. The order of picture blocks remained constant across all participants, with half of the subjects per group starting with a Maintain and half starting with a Reappraisal block.

Pictures were presented in eight blocks of five images, one after another for 5 s each, separated by 1-s blank screen interstimulus intervals. Picture blocks alternated with grey fixation baseline blocks of 30 s, and experiments started with a baseline block. For half of the blocks, participants were instructed to passively view the images and naturally experience the emotional state evoked, without attempting to regulate or downregulate the provoked negative affect by using strategies of cognitive reappraisal (for example, reframe, rationalising). These strategies were taught before the scan using different images. Instructions were given by presenting the word Maintain or Reappraise on screen for 4 s before a block. At the end of each picture block, a 4-point rating scale

have a key role in maintaining the disorder and are targeted during exposure-based cognitive-behaviour therapy (CBT).\textsuperscript{30,41} Experimental research has confirmed that during the presentation of threatening images, anxious participants are more likely to use dysfunctional regulation strategies such as suppression or cognitive avoidance. In contrast, they are less likely to use adaptive, successful techniques such as positive reappraisal.\textsuperscript{32,33} In further support of the idea of anxiety disorders being associated with an increase rather than a decrease in neural responses associated with affect regulation, CBT has been shown to lead to a decrease in responsivity in prefrontal brain areas usually implicated in cognitive control, such as the dlPFC and vmPFC.\textsuperscript{24,34,35} These results question whether anxiety involves increased or decreased engagement of prefrontal areas involved in cognitive control, and how such activation patterns are functionally connected with activation in limbic areas of the fear circuit. This study therefore aimed to assess regional neural correlates of emotion regulation in unmedicated patients with panic disorder compared with healthy volunteers, and functional connectivity between amygdala and prefrontal areas thought to be implicated in cognitive control. We therefore used a well-established emotion regulation paradigm\textsuperscript{12,36} that allows assessment of neural
Reappraisal × time series and the regressors of no interest (instructions and ratings). These individual contrast images were then entered into the group level, using a mixed-effects analysis across the whole brain, in order to identify brain areas that showed activity that covaried stronger with that of the left and right amygdala in one of the two groups during Maintain blocks, Reappraisal blocks or picture blocks in general. Pearson’s correlations were computed for standardised betas (extracted from significant clusters) and panic symptom severity (calculated as the mean of the scores achieved on the Agoraphobic Cognitions Questionnaire and Body Sensations Questionnaire).

Given previous research indicating increased amygdala–dmPFC connectivity during the anticipation of threat56,57 and in anxiety disorders,58 strength of coupling between the amygdala seed regions and the dmPFC was identified using a ROI approach. We extracted regression standardised beta values reflecting coactivation between the amygdala seeds and a 10-mm radius drawn around 12/42/54, a previously published peak voxel of a dmPFC cluster relevant in emotion regulation using an identical task and instructions,13 and entered these into group × task ANOVAs and Pearson’s correlation analyses (panic severity).

Voxel-based morphometry. Voxel-based morphometry was carried out to be able to add grey matter maps as covariates to the functional MRI (fMRI) analysis model, to only identify group differences in functional activation that reflect cognitive-emotional rather than grey matter differences. Brain extraction and tissue-type segmentation were performed and resulting grey matter partial volume images were aligned to standard space using first linear (FLIRT) and then non-linear (FNIRT) registration tools. The resulting images were averaged, modulated and smoothed with an isotropic Gaussian kernel of 7 mm full width at half maximum to create a study-specific template, and the grey matter images were re-registered to this, including modulation by the warp field Jacobian. Threshold-free-cluster-enhancing correction was applied. Finally, voxel-wise general linear modelling was applied using permutation nonparametric testing (5000 permutations), correcting for multiple comparisons across space.

RESULTS

Affect ratings and behavioural data

Mood and anxiety measures. Patients reported significantly higher trait anxiety and depression (Hospital Anxiety and Depression Scale) levels than controls, and they showed more fear of physical sensations (Body Sensations Questionnaire) (Table 1). With respect to state mood, there were significant group differences on all Visual Analogue Scales taken before and after the scan (Table 1). Patients showed lower anxiety scores and higher calm scores after the scan compared with baseline (both t = 3.20, both P < 0.005).

HRV and negative affect ratings during scan. Differences were analysed performing group × task mixed-model ANOVAs. In patients but not controls, LF/HF ratio was higher in Maintain compared with Reappraisal blocks (Table 1; group × task F = 5.89, df = 1/26, P = 0.023, d = 0.83; paired-samples t-test t = 2.14, df = 14, P = 0.049). During Maintain blocks, patients showed higher LF/HF ratios than controls (independent samples t = 2.09, df = 26, P = 0.047, d = 0.82), suggesting reduced HRV and more sympathetic compared with parasympathetic activation. Groups were not significantly different in LF/HF ratio during Reappraisal (t = 0.52, df = 26, P = 0.606).

Negative affect ratings were lower in Reappraisal versus Maintain blocks in both groups, without between-group differences (Table 1; Task F = 4.52, df = 1/34, P < 0.001; group × task both F < 0.04, both P > 0.855).

BOLD fMRI

Whole-brain analysis

Main effect of task (Reappraise versus Maintain, across groups): Reappraisal was associated with increased activation in bilateral dorsal ACC, dmPFC, dIPFC, vlPFC, angular gyri, superior lateral occipital cortices, orbitofrontal cortices/subcortical...
Main effect of group (picture blocks versus baseline): Compared with controls, patients showed significantly higher activation in bilateral dmPFC and dIPFC, left dorsal ACC and right supplementary motor area, as well as left inferior frontal gyrus, left middle temporal gyrus, left inferior and superior lateral occipital cortex, left occipital fusiform gyrus and left angular gyrus during the eight picture blocks versus the fixation screen baseline (Figure 1b; Table 2B; all \( d > 1.19 \)). Percent signal change in these clusters was...
Figure 1. Whole-brain fMRI results. All images thresholded at $Z > 2.3$, $P < 0.05$, corrected. (a) Main effect of task: across both groups, Reappraisal (versus Maintain) led to greater BOLD signal response in bilateral dorsal anterior cingulate cortex, dorsomedial, dorsolateral, and ventrolateral PFC, orbitofrontal cortex, lateral occipital cortex, angular gyrus, cerebellum and occipital fusiform and inferior temporal gyri, and left middle temporal gyrus, and to a decrease in activation in bilateral precuneus ext. lingual gyrus. (b) Main effect of group: compared with controls, patients showed increased activation in prefrontal, temporal and occipital areas, including bilateral dorsomedial and dorsolateral PFC, left dorsal anterior cingulate cortex, right supplementary motor area, left inferior frontal and middle temporal gyrus, left lateral occipital cortex and occipital fusiform gyrus, and left angular gyrus during picture blocks (versus fixation baseline block). (c) Group × task interaction: maintaining negative affect (versus Reappraisal) was associated with increased signal response in patients compared with controls in a right frontal pole cluster including the vlPFC, vmPFC and dmPFC (top panel), and a limbic cluster including parts of the right hippocampus, posterior cingulate cortex, precuneus and lingual gyrus (bottom panel). In controls, Maintain (versus Reappraisal) was related to decreased activation in this limbic cluster. For both clusters, BOLD% signal change during Maintain minus Reappraisal blocks was significantly correlated with symptom severity in patients. MNI coordinates refer to the peak activation voxel of the cluster and main sub-regions within the same cluster (significant group differences are in bold).

Table 2. (A) Areas of significant increase and decrease in BOLD response during voluntary emotion regulation (Reappraisal versus Maintain) across both groups. Areas of significant increase in BOLD response in patients versus controls during (B) picture blocks versus fixation baseline blocks, and (C) Maintain versus Reappraisal blocks. MNI coordinates refer to the peak activation voxel of the cluster and main sub-regions within the same cluster (significant group differences are in bold).

| BA Side Cluster size (voxels) | MNI (x, y, z) | Z-score | P-score |
|-------------------------------|---------------|---------|---------|
| **(A) Main effect of task across groups** | | | |
| Increased activity during reappraisal ($R > M$) | | | |
| Dorsal ACC ext. dorsomedial PFC | 6/8/32 L | 14,701 | −4,20,50 | 5.86 < 0.001 |
| Dorsal ACC ext. dorsomedial PFC | 6/8/32 R | | 4,28,40 | 4.99 |
| Ventrolateral ext. dorsolateral PFC | 9/45/46 L | | 36,46,12 | 4.9 |
| Cerebellum ext. occipital fusiform/inferior temporal gyrus | 19/37 L | 7,274 | −36, −64, −28 | 4.30 < 0.001 |
| Middle temporal gyrus | 20/21 L | | −60, −32, 0 | 4.2 |
| Angular gyrus | 39/40 L | | 44, −60,42 | 4.18 |
| Superior lateral occipital cortex | 5/7 L/R | | 0, −68,68 | 4.06 |
| Angular gyrus | 39/40 L | | −60, −44,34 | 4.06 |
| Cerebellum ext. occipital fusiform/inferior temporal gyrus | 19/37 R | 2,184 | 36, −60, −52 | 4.46 < 0.001 |
| Fronto orbital cortex | 11/38 L | 544 | 16,22, −16 | 3.92 0.006 |
| Subcallosal cortex ext. caudate | 25 L | | 6,20, −12 | 3.89 |
| Subcallosal cortex ext. caudate | 25 L | | −4, −10 | 3.25 |
| Decreased activity during reappraisal ($M > R$) | | | |
| Precuneus ext. lingual gyrus | 19 R | 477 | 26, −32,22 | 3.67 0.014 |
| Precuneus ext. lingual gyrus | 19 L | 434 | −20, −42,24 | 3.90 0.024 |
| **(B) Pictures vs baseline/patients vs controls** | | | |
| Dorsal anterior cingulate cortex | 32 L | 1,352 | −8,28,38 | 3.99 < 0.001 |
| Dorsomedial PFC | 8/9 L | | −24,38,46 | 3.65 |
| Dorsomedial PFC | 8/9 R | | 4,42,36 | 3.56 |
| Supplementary motor area | 6 L/R | | 4,14,72 | 3.55 |
| Dorsolateral PFC | 45 L | 799 | −46,30,28 | 3.90 < 0.001 |
| Inferior frontal gyrus | 44/48 L | | −54,16,14 | 3.88 |
| Dorsolateral PFC | 46 L | | −40,34,26 | 3.65 |
| Dorsolateral PFC | 9 L | | −42,18,44 | 3.50 |
| Middle temporal gyrus ext. inferior lateral occipital cortex | 20/21 L | 535 | −56, −28, −12 | 3.77 0.003 |
| Occipital fusiform gyrus | 37 L | | −48, −66, −12 | 3.53 |
| Inferior lateral occipital cortex | 19 L | | −44, −74, −14 | 3.33 |
| Superior lateral occipital cortex | 7 L | 493 | −32, −68,40 | 3.97 0.006 |
| Angular gyrus | 40 L | | −50, −52,54 | 3.61 |
| Dorsomedial PFC | 9 R | 459 | 20,36,54 | 3.30 0.009 |
| Dorsolateral PFC | 44 R | | 50,18,38 | 3.28 |
| Dorsolateral PFC | 8/9 R | | 42,18,50 | 3.19 |
| **(C) Maintain vs reappraise/patients vs controls** | | | |
| Ventrolateral PFC | 46/47 R | 417 | 48,56, −2 | 3.98 0.030 |
| Ventromedial/dorsomedial PFC | 10/11 R | | 32,68,2 | 3.15 |
| Hippocampus ext. precuneus/posterior cingulate | 27/29/30/37 R | 387 | 14, −42,4 | 3.80 0.044 |
| Hippocampus | 27 R | | 24, −34, −12 | 3.65 |
| Lingual gyrus | 37 R | | 26, −42, −8 | 3.16 |
| Precuneus | 30 R | | 12, −52,10 | 3.04 |

Abbreviations: ACC, anterior cingulate cortex; BA, Brodmann area; ext., extending into; L, left; M > R, Maintain versus Reappraisal; MNI, Montreal Neurological Institute; PFC, prefrontal cortex; R, right; R > M, reappraisal versus maintain.
not correlated with panic symptom severity in any of the groups (all $r < 0.26$, $P > 0.293$).

Group × task interaction (Maintain versus Reappraise): Results showed a significant group × task interaction in a frontal pole cluster spanning the right vPFC and the right dmPFC and vmPFC, as well as a limbic cluster including parts of the right dorsal hippocampus and posterior cingulate, precuneus and lingual gyrus (Figure 1c; Table 2C). Post hoc analyses on BOLD signal change extracted from each of the two clusters indicated that the PFC interaction was driven by a group difference in response to pictures during Maintain blocks (ANOVA task × group $F = 14.79$, df = 1/34, $P = 0.001$, $d = 1.32$), with patients showing significantly higher activation than controls (Maintain $t = 2.77$, df = 34, $P = 0.009$, $d = 0.80$; Reappraise $t = 1.66$, df = 34, $P = 0.119$, $d = 0.55$). The group × task interaction in the hippocampal cluster was based on patients showing an increase in activation in this area in the Maintain condition and a decrease during Reappraisal (group × task interaction $F = 14.83$, df = 1/34, $P < 0.001$, $d = 1.32$; post hoc $t$-tests Maintain $t = 2.05$, df = 34, $P = 0.048$, $d = 0.63$; Reappraise $t = 2.93$, df = 34, $P = 0.006$, $d = 1.14$). In patients but not controls, BOLD activation in both these clusters during Maintain minus Reappraisal blocks was positively correlated with panic severity (PFC: patients $r = 0.49$, $P = 0.038$, controls $r = 0.36$, $P = 0.142$; hippocampus: patients $r = 0.49$, $P = 0.038$; controls $r = -0.23$, $P = 0.369$). However, these do not survive conservative Bonferroni correction (that is, required $P < 0.025$).

ROI analysis: A hemisphere × group × task ANOVA for the BOLD percent signal change extracted for the left (Maintain: patients $0.40 ± 0.44$, controls $0.15 ± 0.17$; Reappraise: patients $0.11 ± 0.36$, controls $0.19 ± 0.22$) and right amygdala (Maintain: patients $0.37 ± 0.55$, controls $0.13 ± 23$; Reappraise: patients $0.10 ± 0.37$, controls $0.17 ± 0.24$) spheres revealed a significant group × task interaction ($F = 6.82$, df = 1/34, $P_{\text{Bonferroni}} = 0.026$, $d = 0.90$), without any laterality differences (all $F < 0.10$, all $P_{\text{Bonferroni}} > 0.998$). This effect was driven by patients showing higher activation than controls in Maintain blocks ($t = 2.43$, df = 34, $P_{\text{Bonferroni}} = 0.042$, $d = 0.81$). In patients ($r = 0.42$, $P = 0.043$) but not controls ($r = -0.13$, $P = 0.613$), Maintain minus Reappraisal BOLD percent signal change in the amygdala was positively correlated with panic symptom severity.

**Connectivity analyses**

Whole-brain results: In patients versus controls, activity in each amygdala during picture blocks (versus baseline) was significantly more strongly correlated with activity in the left and right occipital cortices, occipital poles, occipital fusiform gyri and lingual gyri (right amygdala: right (R) cortical cluster: 591 voxels, MNI $Z = 3.86$, $d = 0.84$; left amygdala: R cortical cluster: 578 voxels, MNI $Z = 3.67$; L cortical cluster: 461 voxels, MNI $Z = 3.47$). In patients but not controls, the magnitudes of right amygdala–occipital clusters and left amygdala–occipital clusters coupling were positively correlated with symptom severity (patients: L/R amygdala–occipital clusters both $r > 0.42$, both $P < 0.042$; controls: L/R amygdala–occipital clusters both $r > 0.35$, both $P > 0.078$) (Figure 2). Amygdala–dorsomedial PFC coupling: For the right amygdala seed, we found a significant group × task interaction driven by patients showing higher right amygdala–right dmPFC connectivity than controls during Maintain blocks (ANOVA group × task $F = 7.17$, df = 1/34, $P_{\text{Bonferroni}} = 0.022$, $d = 0.92$; $t$-test $t = 2.39$, df = 34, $P_{\text{Bonferroni}} = 0.046$, $d = 0.80$). For the left amygdala seed, the
ANOVA yielded no group x task interaction ($F = 0.27$, $df = 1/34$, $P_{\text{Bonferroni}} = 0.904$). In patients but not controls, the magnitudes of right amygdala–dmPFC coupling and left amygdala–dmPFC coupling during Maintain minus Reappraisal blocks were positively correlated with panic symptom severity (patients: L/R amygdala–dmPFC clusters both $r > 0.55$, both $P < 0.010$; controls: L/R amygdala–dmPFC clusters both $r < 0.20$, both $P > 0.214$) (Figure 3).

**DISCUSSION**

We believe this is the first study to simultaneously explore regional neurofunctional activation and limbic–prefrontal connectivity in patients with an anxiety disorder during incidental versus intentional emotion regulation. In line with our hypotheses, we found a pattern of increased brain activity in patients compared with controls in both limbic and prefrontal areas during incidental emotion regulation (Maintain) and in response to images in general. These differences were reduced, or even reversed, during intentional regulation (Reappraisal). The differences in brain activity were accompanied by significantly reduced heart rate variability in patients versus controls during incidental regulation only, highlighting patients’ ability to regulate emotional response given appropriate cognitive strategies. Although these results challenge influential models of fear that propose impaired allocation of PFC resources as a neurobiological basis for the development of an anxiety disorder, they are well in line with more recent frameworks.

In the neurobiological hypervigilance-avoidance model, Hoffman et al. postulate that threat processing in anxiety is associated with two different sets of functional activation patterns: hypervigilance processes and maladaptive, avoidant emotion regulation processes. The hypervigilance processes are thought to include amygdala hyperactivation in response to the detection of threat, which in turn facilitates visual processing in the occipital cortex. This is thought to enhance processes of selective attention and monitoring in the dmPFC while recruiting the hippocampus to provide information about memory associations with the potential threat stimulus. The maladaptive regulation processes are thought to include hyperactivation in a range of ventral and lateral prefrontal–cortical regions known to be implicated in regulating negative emotional reactivity.

In line with the assumed hypervigilance circuit, our patients showed increased activation in occipital areas, dorsal mPFC and ACC, and increased amygdala–occipital connectivity when viewing threat images in general. They also demonstrated amygdala and hippocampus hyperactivation and increased amygdala–dmPFC connectivity during Maintain (versus Reappraisal) blocks. Most of these parameters correlated positively with symptom severity.
severity. These results also fit in well with other recent imaging studies, suggesting that threat processing in anxiety disorders is associated with increased activity in amygdala, hippocampus12,23,26,60,61,62 and occipital cortex,27,62 or in dorsal ACC and mPFC17–22 areas that have been implicated in selective attention, threat bias and monitoring.56,63–68

The results are also in line with prior studies demonstrating increased functional amygdala-dmPFC connectivity during the anticipation of threat in healthy volunteers.56,57 with additional increases in subjects with higher trait anxiety, neuroticism or anxiety disorders.56–58 Furthermore, increased attentional bias magnitude derived from behavioural tasks has been shown to be correlated with increased amygdala-dorsal ACC connectivity in healthy volunteers,69 providing further evidence that such brain activation patterns might predispose patients to selectively focus their attention to threat information in their environment. The potential role of these proposed areas of hypervigilance in the psychopathology of anxiety disorders is further supported by clinical research showing a reduction of activation in amygdala,17,70 hippocampus,24 dorsal mPFC and ACC17 following successful CBT.

The assumption that hyperactivation in lateral and ventral prefrontal–cortical regions might reflect anxiety-specific dysfunctional regulation strategies is supported by our findings of patients showing increased activation in dorsal and ventral lateral PFC in response to threat images in general. Furthermore, they showed increased response in vmPFC and vIPFC during incidental regulation, with activation strength correlating positively with panic severity. Lateral prefrontal activation has previously been implicated in intentional emotion regulation11,71,72 and inhibitory control73,74 and the vmPFC has particularly been associated with automatic conflict and emotion regulation75–77 in healthy volunteers. In line with our observations, other imaging studies exploring threat processing in anxiety disorders have reported heightened activation in the dlPFC,23,24 vIPFC,20,21,25 and vmPFC,26,27 and CBT has been shown to significantly reduce such hyperactivation.34,35

Taken together, our results provide evidence in favour of recent models of anxiety, which propose that psychopathology might be underpinned by hyperactivation in both limbic and prefrontal–cortical brain regions in response to threat.28,29 Such findings contradict previous frameworks postulating that impaired emotion regulation in anxiety is correlated with reduced recruitment of PFC areas of top–down control.13,14 Strikingly, these early models were greatly based on research investigating neural processing in high trait anxiety or post-traumatic stress disorder (PTSD), which appear to characteristically be associated with decreased recruitment of PFC resources during threat processing.29 Neurobiological activation patterns in high trait anxiety in response to threat might still be adaptive and as such distinctive from activation patterns in anxiety disorders. Notably, PTSD is not classified as an anxiety disorder in DSM-V (APA, 2013) anymore, as the key symptom is the re-experience of a de facto trauma rather than arbitrary fear, and as fear is not the only and not necessarily the predominant emotion.78,79 It is also plausible that these differences in aetiology and symptomatology between PTSD and anxiety disorders might be underpinned by differences in neurobiological pathophysiology, and future research will have to address these issues explicitly. It is also possible that differences in paradigms used between studies might contribute to contrary findings in prefrontal–cortical activation, with previous studies in trait anxiety often tapping into rapid resolution of emotional conflict11 rather than emotion processing and regulation per se.

It might appear puzzling why we rarely found group differences in functional activation for reappraisal blocks. However, reappraisal is one of the key strategies taught during CBT56 to which patients with panic disorder have been shown to be particularly responsive, even after only one treatment session.53 It is possible that having trained patients to successfully use reappraisal before scanning has provided them with sufficient, healthy regulation strategies. Furthermore, our results allow no final conclusions with respect to the exact role of the panic-specific processing patterns observed here in the onset and maintenance of an anxiety disorder. Future studies will have to establish whether these patterns of activity are sensitive to treatment, and whether any changes in these parameters are causally related to clinical improvement, which would confirm their proposed role as key mechanisms in the pathogenesis of anxiety.

CONFLICT OF INTEREST

CJH has valueless shares in p Vital and serves on their advisory panel. She has received consultancy payments from p Vital, Servier, Eli-Lilly, Astra Zeneca, Lundbeck and is a director of Oxford Psychologists Ltd. The remaining authors declare no conflict of interest.

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