# The dorsal medial prefrontal (anterior cingulate) cortex–amygdala aversive amplification circuit in unmedicated generalised and social anxiety disorders: an observational study

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## Summary

**Background** In four previous studies, we have delineated the role of positive circuit coupling between the dorsal medial prefrontal (anterior cingulate) cortex and the amygdala during aversive processing in healthy people under stress. This translational circuit—the aversive amplification circuit—is thought to drive adaptive, harm-avoidant behaviour in threatening environments. We assess the role of this circuit in the pathological manifestation of anxiety disorders.

**Methods** For this single-site study, 45 unmedicated participants (22 with generalised and/or social anxiety disorder and 23 healthy controls) were recruited via advertisements from the metropolitan area of Washington, DC (USA). People who applied to participate in the study had to pass an initial telephone screen and comprehensive screening by a clinician at the National Institutes of Health (NIH; Bethesda, MD, USA). People with a contraindicated medical disorder, past or current psychiatric disorders other than anxiety disorders, and those using psychoactive medications or illicit drugs were excluded. Eligible individuals could participate as either a healthy control or a patient, depending on diagnosis. They were asked to use a button box to complete a simple emotion identification task (fearful *vs* happy faces; 44 trials of each) during functional MRI at the NIH. Functional imaging analysis consisted of event-related activation analysis and psychophysiological interaction connectivity analysis of regions coupled with the amygdala during task performance.

**Findings** A diagnosis-by-valence interaction was recorded in whole-brain amygdala connectivity within the dorsal medial prefrontal (anterior cingulate) cortex clusters identified in our previous study, driven by significantly increased circuit coupling during processing of fearful faces versus happy faces in anxious, but not healthy, participants. Importantly, and in accordance with contemporary theoretical approaches to psychiatry, circuit coupling correlated positively with self-reported anxious symptoms, which provides evidence of a continuous association between the circuit and subjective symptoms.

**Interpretation** In this study and our previous work, we track the functional role of one neural circuit from its involvement in adaptive threat biases under stress, to its chronic engagement in anxiety disorders in the absence of experimentally induced stress. Thus, we uniquely map a mood and anxiety-related circuit across its adaptive and maladaptive stages. Clinically, this study could provide a step towards a more mechanistic continuum-based approach to anxiety disorder diagnosis and might ultimately lead to more targeted treatments for patients with anxiety disorders.

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## Introduction

Pathological anxiety is a large and increasing global health problem.[1] People with the disorder go through periods of crippling anxiety that adversely affect their daily lives. One of the key contributing symptoms is a persistent and debilitating focus upon negative or potentially threatening life experiences.[1] This negative affective bias can be experimentally quantified as increased threat processing at the neural, psychological, and behavioural levels.[1]

However, anxiety can also be an adaptive process that improves an individual's ability to avoid harm. Indeed, negative affective biases towards threats are recorded in healthy people experiencing transient anxiety or stress.[1] The adaptive and maladaptive anxiety-driven negative affective biases might be linked, perhaps falling at opposite ends of the same scale. Such an association would have implications for how we diagnose and treat these disorders, but evidence is scarce at present. This study therefore extends four of our previous studies mapping the circuit-based interactions between the dorsal medial prefrontal (anterior cingulate) cortex and the amygdala during adaptive threat processing in stressed healthy people to address pathological anxiety.

The role of the amygdala in threat processing is well known,[2–4] but the various regions of the brain do not respond in isolation—rather, they constitute nodes in

complex neural circuits.[5,6] Our recent work has therefore begun to outline how interactions between the amygdala and higher cortical regions contribute towards threat-processing biases. In particular, our work suggests that interactions between the dorsal medial prefrontal (anterior cingulate) cortex and amygdala constitute an aversive-amplification circuit whereby increased positive coupling between these regions is associated with increased threat processing under stress.[7–9] Notably, this role, derived from translational animal research,[10] is thought to be distinct from a more frequently studied reciprocal, opposing inhibitory role[11,12] of adjacent ventral (and subgenual) regions of the prefrontal and cingulate cortex (discussed in more detail elsewhere[13–15]).

Specifically, we have shown that stress induced by a threat of shock in healthy people (a manipulation in which participants are told they might potentially receive a shock and that reliably increases psychological, physiological, cognitive, and neural concomitants of stress[1]) drives increased attentional bias (in a face emotion identification task) to fearful faces[16] as a function of increased positive functional connectivity between the dorsal medial prefrontal (anterior cingulate) cortex and the amygdala (figure 1).[7] Second, we have replicated this finding using a different technique, task-independent resting state fMRI; enhanced positive endogenous connectivity (ie, oscillatory connectivity during rest periods with no task) is recorded between the dorsal medial prefrontal (anterior cingulate) cortex and the amygdala during prolonged periods of threat of shock in an adapted resting-state paradigm in healthy participants.[9] Third, we have shown that threat of shock leads to cognitive disturbances in working memory in healthy people[1] that are also associated with increased coupling within this circuit (Vytal K, unpublished). Finally, we have reported that mimicking a pharmacological symptom of anxiety in healthy individuals—reduced serotonergic function—engages functional connectivity within this same circuit during processing of fearful faces.[8] This fourth study provides a putative mechanism by which selective serotonergic reuptake inhibitor drugs might, through modulation of this circuit, alleviate anxiety.[8]

Thus, we have comprehensively mapped the workings of this circuit in healthy people. Therefore, the main goal of the present study is to provide experimental evidence that this functionally mapped circuit might have a key role in pathological anxiety disorders. Previous work focusing on threat processing in anxiety disorders has shown abnormal activity in regions of the prefrontal cingulate cortex and the amygdala.[17–19] However, studies so far have mostly assessed individual region activations (ie, the change in the activity of a region in one condition *vs* another), and not circuitry (ie, the extent to which activity correlates between two regions in one condition *vs* another). Correlation between regions is thought to at least partly represent flow of information between them, and can be seen in the absence of activation changes (for more details, see elsewhere[20]). Such between-region
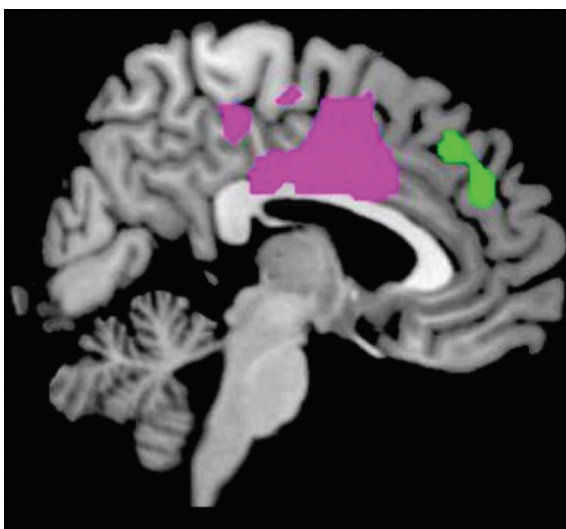


*Figure 1:* **Regions of interest derived from our study using threat of shock as a stress induction in healthy participants**[7]
Both a posterior cluster encompassing dorsal anterior cingulate and medial prefrontal cortex (dorsal region highlighted in dark pink) and a rostral cluster in the dorsal medial prefrontal cortex (rostral region highlighted in green ) showed increased positive connectivity with the amygdala during the processing of fearful faces under stress and were used to create regions of interest for the present study. These regions of interest are freely available to download online.

interactions therefore provide better insight into the way these regions act as a circuit.

Psychiatric disorders are increasingly recognised to be unlikely to fall within the categorical (ie, healthy/unwell) diagnoses of existing diagnostic criteria, but rather lie along a scale from more "normal" to more "impaired" function (for more details, see elsewhere[21]). Our previous studies showed that activation of the dorsal medial prefrontal (anterior cingulate) cortex and amygdala circuit falls along a continuous dimension as a function of trait anxiety symptoms,[7,9] with increased trait feelings of anxiety (a vulnerability factor for anxiety disorders) being associated with greater positive coupling within this circuit. This finding generates a secondary prediction: pathological anxiety symptoms will also fall along this continuum. This theory suggests that anxiety symptoms that are severe enough to interfere with daily living should be associated with even more circuit engagement along the same dimensional index. Such a finding would, from a clinical perspective, help to refocus our understanding of anxiety disorders away from discrete diagnoses and towards more of a range or scale.

## Methods
### Study design and participants
We did this single-site study at the National Institutes of Health (NIH) Clinical Center in Bethesda (MD, USA). Participants were recruited from the Washington DC metropolitan area (USA) for the study by flyers and advertisements placed in local newspapers. One line of recruitment sought participants who had anxiety

For **regions of interest** see http://figshare.com/authors/ Oliver_Robinson/568652

| | Anxiety disorders (n= 22) | Healthy controls (n=23) | F test value | p value |
|---|---|---|---|---|
| **Demographics** | | | | |
| Female sex | 16 (73%) | 14 (61%) | .. | .. |
| Age (years) | 28 (8) | 28 (6) | 0·04 | 0·9 |
| **Questionnaire measures on day of scan** | | | | |
| WASI | 118 (10) | 119 (10) | 0·2 | 0·6 |
| STAI state | 40 (12) | 25 (4) | 33 | <0·0001 |
| STAI trait | 49 (12) | 26 (5) | 71 | <0·0001 |
| BDI | 9 (9) | 0·7 (1) | 21 | <0·0001 |

Data are n (%) or mean (SD). All participants were right handed except for one patient and one healthy control who were left handed. WASI=Wechsler Abbreviated Scale of Intelligence. STAI=State-Trait Anxiety Inventory. BDI=Beck Depression Inventory.

*Table 1:* Baseline characteristics

problems whereas another line did not specify psychiatric issues. Following an initial telephone screen, participants visited the NIH for comprehensive screening by a clinician, which comprised a physical examination, urine screen, and a Structured Clinical Interview [SCID] from the Diagnostic and Statistical Manual of Mental Disorders [DSM], Fourth Edition.[22] Exclusion criteria were: contraindicated medical disorder; past or current psychiatric disorders other than anxiety disorders; and use of psychoactive medications or illicit drugs (as tested by the urine screen). People who passed this screening stage were given the option to participate in the study as either a healthy control or a patient (depending on their diagnosis). Participants completed measures of anxiety (State-Trait Anxiety Inventory [STAI][23,24]), depression (Beck Depression Inventory [BDI][24]), and intelligence quotient (Wechsler Abbreviated Scale of Intelligence [WASI][25]). Five patients were excluded because of scan acquisition artifacts (eg, caused by extreme movements or scanner malfunction); therefore, the final sample consisted of 45 unmedicated people, 22 of whom were suffering from a current anxiety disorder. 15 of these patients had a generalised anxiety disorder, of which nine were comorbid with a secondary diagnosis of social anxiety disorder; and seven had a social anxiety disorder. 23 healthy controls also participated. In the patients with anxiety disorders, the mean estimated illness duration was 16 (SD 8) years. Seven patients had received previous pharmacological treatment, which had been discontinued (>10 years ago [n=5], >6 months ago [n=1], or >2 months ago [n=1]). Unmedicated status was necessary to avoid potential drug-linked vascular confounders. Patients and healthy controls were matched for demographic variables (table 1). All participants provided written informed consent that was approved by the Combined Neuroscience Institutional Review Board of the NIH.

## Procedures

For their task in this study, participants were asked to use a button box to identify whether faces[16] were fearful or happy. The task consisted of 88 trials (44 fearful faces and 44 happy faces) with 2000–4000 ms jitter between the trials. Each stimulus was presented for 990 ms and 30 s of fixation was presented at the start and end of the task. This task is the same as that used previously in healthy controls[7] but without the concurrent threat of shock stress manipulation used in that previous study. Participants were asked to respond as quickly as possible using a button box placed upon their abdomen in the scanner. The task was projected on a screen to the rear of the scanner, visible by means of a mirror attached to the head coil (an integral component of the fMRI scanner).

We used a 3T Skyra scanner (Siemens, Malvern, PA, USA) to acquire one 207 volume acquisition echo planar imaging (EPI) sequence (flip angle 70°; repetition time [TR] 2000 ms; echo time [TE] 30 ms; field of view [FOV] 100 cm; slice thickness 3 mm; matrix 64×64 samples sagittal). We discarded the first five volumes from each run to allow for scanner equilibration. The structural sequence comprised a magnetisation-prepared rapid gradient echo anatomical reference image (flip angle 9°; TR 1900 ms; TE 2·1; inversion time 450 ms; FOV 100 cm; slice thickness 0·9 mm; matrix 256×256). We preprocessed and analysed images using SPM version 8. SPM refers to the "conjoint use of the general linear model (GLM) and Gaussian random field (GRF) theory to analyse and make classical inferences about spatially extended data through statistical parametric maps (SPMs)" (for SPM software information, see online).

## Statistical analysis

Our analyses consisted of five well-established steps, the first of which was preprocessing to transform the blood-oxygen level-dependent (BOLD) signal acquired during scanning into the same standardised space across time and across all participants. Step two was statistical activation analysis to generate BOLD signal activation estimates for each trial type (fearful *vs* happy faces) for each participant (first-level event-related analysis with use of mass univariate general linear models). The third step was statistical connectivity analysis to estimate which regions across the whole brain are significantly associated with the BOLD activity recorded in the amygdala during each trial type (a psychophysiological interaction analysis). Fourth was a group-level analysis in which summary estimates of activation or connectivity for each participant are compared across groups (second-level analysis with *t* tests). The fifth step was a group-level continuous variable analysis in which summary estimates of activation or connectivity for each participant are correlated with individual difference measures (eg, trait personality scales or mean reaction times).

Preprocessing consisted of within-participant realignment, coregistration, segmentation, normalisation, and smoothing (with a Gaussian kernel 8 mm full width at half maximum). In an event-related analysis, we used a

general linear model to estimate the BOLD signal change associated with the onset times of each face valence (fearful or happy). We also included motion parameters created during the realignment phase as nuisance regressors in this model (to account for noise associated with participant movement). This process was repeated for each participant and asks: what is the BOLD signal change associated with fearful and happy faces?

We then created a generalised psychophysiological interaction general linear model for each participant in the same manner as in our previous studies[7,8] (which were distinct studies for which the sample populations were recruited separately). Specifically, we used SPM8 code to generate the following regressors from the event-related model described above: an eigenvariate summary of BOLD signal localised within spatial confines of the amygdala seed used in our previous study[7] (an anatomical region of interest [ROI] defined by an automated anatomical labelling library[26]) across time; separate psychological regressors representing the onsets of each happy face and each fearful face; and psychophysiological interaction terms representing the interaction between the first two regressors. Next, we created a general linear model for each participant in which we included one regressor representing a deconvolved BOLD signal alongside each psychological and psychophysiological interaction term for each event type. This model therefore asks: for each participant, which regions of the brain show a BOLD signal that is significantly associated with that of the amygdala during the events of interest (fearful or happy)? This is the circuit-coupling measure that we are interested in.

For each participant, we then created a contrast representing the regions across the whole brain that were more strongly related with the amygdala during fearful face processing than during happy face processing (ie, fearful *vs* happy contrast). We compared these within-participant contrasts at the group level in a standard SPM8 two-sample (healthy *vs* anxious) *t* test. This analysis provided us with an estimate of regions, across the whole brain, that showed greater correlation with the amygdala in anxious patients than in healthy controls during fearful (compared with happy) face processing (ie, a diagnosis-by-valence interaction). We did similar analyses for event-related activations of each trial versus baseline.

To directly compare cross-study activation with our previous study, we created a priori ROIs from the clusters in our first study[7] (figure 1) using the "get SPM cluster" function of the MARSBAR toolbox for SPM8.[27] These clusters were generated at a threshold of p<0·001 (uncorrected) in our previous data from the within-participant, whole-brain threat-by-valence interaction map generated by use of the flexible factorial model in SPM8 (for more details, see the original report[27] or download the ROIs from the internet). This ROI comprised the largest, more dorsal and posterior peak dorsal medial prefrontal cortex–dorsal anterior cingulate
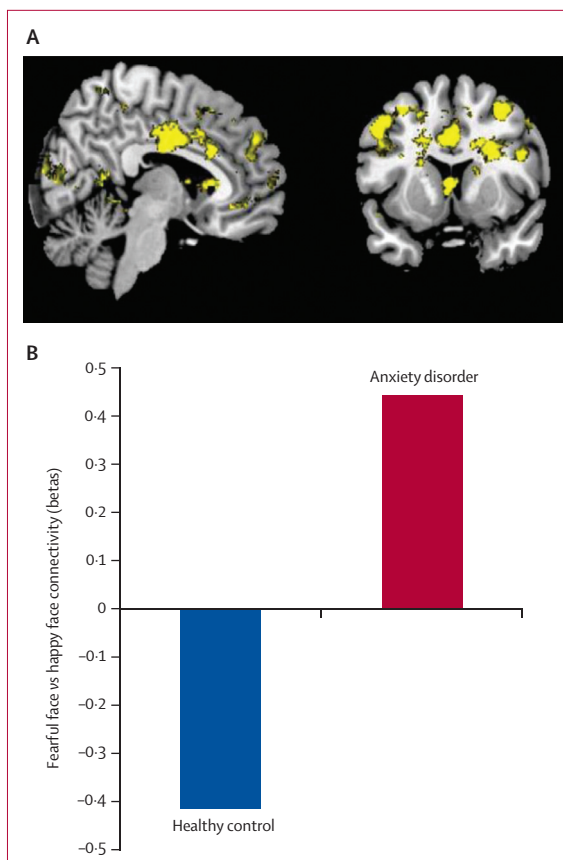


*Figure 2:* Categorical analysis
(A) A whole-brain diagnosis-by-valence interaction in dorsal anterior cingulate cortex–amygdala connectivity was recorded with yellow areas indicating increased circuit activity during observation of fearful versus happy faces in patients. (B) Fearful face versus happy face connectivity in healthy controls and patients with anxiety disorders; data extracted for illustrative purposes from the a priori peak (x,y,z=2,2,40).

cortex cluster (referred to as dorsal from now on) and a more rostral dorsal medial prefrontal cortex area (referred to as rostral from now on). Activations that fall within these regions in the group analyses of the present study can be said to overlap with the activation in our previous study.

We report Montreal Neurological Institute (MNI) standardised coordinates[28] (denoted as x,y,z). For additional corroboration, we extracted the activation and connectivity estimates (betas) from the peak voxel (x,y,z=2,2,40) from our previous study. We then analysed these extracted betas using general linear models in SPSS version 22.

We did continuous variable functional MRI analyses by separately correlating STAI trait anxiety[24] (measured on the day of testing) and behavioural bias (fearful minus happy reaction times) with the fearful face versus happy face connectivity estimates derived from the connectivity analysis. This analysis therefore asks, across the whole sample or within groups, which connectivity estimates (across the whole brain) are significantly associated with the bias or trait anxiety variables?

For **eigenvariate summary** see http://www.fil.ion.ucl.ac.uk/spm/doc.intro

For **ROIs** see http://figshare.com/authors/Oliver_Robinson/568652

| | Cluster size (k) | t test value | Automated anatomical label[26] |
|---|---|---|---|
| 4,–8,32 | 99 | 4·69 | Right dorsal anterior cingulate cortex |
| –4,–6,36 | † | 4·14 | Left dorsal anterior cingulate cortex |
| 6,0,34 | † | 3·68 | Right dorsal anterior cingulate cortex |
| 20,–10,34 | 127 | 4·65 | Right dorsal anterior cingulate cortex |
| 30,–4,40 | † | 4·64 | Right precentral cortex |
| 22,–6,44 | † | 4·62 | Right precentral cortex |
| –28,–14,26 | 46 | 4·58 | Left insula |
| –30,–4,32 | † | 3·93 | Left precentral cortex |
| –26,–22,26 | † | 3·83 | Left caudate |
| –66,–26,2 | 35 | 4·46 | Left middle temporal cortex |
| –16,44,–6 | 17 | 4·39 | Left ventral anterior cingulate |
| –14,–30,48 | 10 | 4·37 | Left dorsal caudal cingulate |
| 34,–62,16 | 14 | 4·29 | Right calacrine fissure |
| –26,16,52 | 38 | 4·19 | Left dorsal medial frontal cortex |
| –18,22,46 | † | 3·43 | Left dorsal medial frontal cortex |
| –12,22,4 | 23 | 4·18 | Left caudate |
| –30,38,–4 | 12 | 4·08 | Left inferior orbitofrontal cortex |
| –52 | 37 | 4·06 | Cerebellum |
| –36,–34,30 | 13 | 4·03 | Left inferior parietal cortex |
| 40,–30,6 | 4 | 4 | Left superior temporal gyrus |
| 28,10,28 | 13 | 3·96 | Left inferior frontal cortex |
| –26,0,26 | 3 | 3·96 | Left caudate |
| 26,–20,32 | 5 | 3·9 | Right caudate |
| 34,–4,38 | 2 | 3·88 | Right precentral cortex |
| 20,12,34 | 3 | 3·84 | Right medial cingulate |
| –10,–26,44 | 4 | 3·82 | Left medial cingulate |
| 0,6,8 | 7 | 3·81 | Left caudate |
| 18,16,14 | 3 | 3·79 | Right caudate |
| –34,–66,6 | 3 | 3·69 | Left medial occipital cortex |
| 0,12,34 | 22 | 3·69 | Left medial cingulate |
| –14,28,8 | 2 | 3·6 | Left caudate |
| –16,50,16 | 6 | 3·6 | Left superior frontal gyrus |
| 48,14,28 | 2 | 3·57 | Right inferior frontal gyrus |
| 42,–14,–10 | 2 | 3·54 | Right insula |
| 26,–52,14 | 2 | 3·54 | Right calcarine fissure |
| 36,–48,34 | 2 | 3·53 | Right inferior parietal cortex |
| –50,24,36 | 5 | 3·52 | Left medial frontal cortex |
| –58 | 6 | 3·46 | Left superior temporal gyrus |
| 14,40,42 | 2 | 3·44 | Right superior frontal cortex |

*Interactions presented are significant at p≤0·001 uncorrected at the whole brain level. †Each of these peaks fall within the larger cluster above.

*Table 2:* **Clusters showing significant connectivity with the amygdala in the diagnosis-by-valence interaction*, by Montreal Neurological Institute coordinates (x,y,z)**

We established upper and lower value boundaries for outliers by use of the formula $Q3 \pm (2 \cdot 2 \times [Q3 - Q1])$,[29] in which Q3 is the third quartile and Q1 is the first quartile. All extreme values fall within the bounds for outliers (–0·4 to 96·4 for trait anxiety and –2·6 to 4·8 for connectivity betas); hence, no participants were excluded. Interactions of interest were significant at $p < 0 \cdot 05$ familywise error rate (FWE), corrected for multiple comparisons, both across the whole brain and within our a priori ROIs. Additional analyses are reported at $p < 0 \cdot 001$ uncorrected when they are of a priori relevance. Findings below these thresholds are judged to be non-significant.

### Role of the funding source
The funder had no input in study design; in the collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit for publication. OJR, LL, KV, PA, and CG had access to all the study data. CG had final responsibility for the decision to submit for publication.

### Results
The diagnosis-by-valence interaction analysis showed, as postulated, a whole-brain peak amygdala connectivity with the dorsal medial prefrontal (anterior cingulate) cortex (peak x,y,z=4,–8,32; $t$=4·69; p[FWE corrected]=0·0289; figure 2A, table 2). Analyses of this connectivity with use of an ROI generated from the largest ROI cluster (dorsal) from our previous study (figure 1) showed significant overlap across studies (peak x,y,z=4,–8,32; $t$=4·43; p[FWE corrected]=0·001). We recorded the same pattern at a non-significant threshold in an ROI generated from the rostral cluster (peak x,y,z=14,40,42; $t$=3·44; p[uncorrected]<0·0006).

Breakdown of this interaction into groups (control/patient) and valence (fear/happy) showed a significant a priori increase in fear versus happy activation in anxious patients (dorsal: peak x,y,z=14,4,36; $t$=4·0; p[uncorrected]=0·0008), but not in healthy controls (dorsal: p[uncorrected]>0·15). Betas extracted from the peak voxel from our previous study (x,y,z=2,2,40) for illustrative purposes showed that this diagnosis-by-valence interaction ($F$[degrees of freedom 1,43]=5·3, p=0·03; figure 2B) was driven by increased coupling during the processing of fearful faces versus happy faces in patients with anxiety disorders ($t$[21]=2·2, p=0·039), but not healthy controls (p=0·2).

This fear versus happy connectivity increased as a function of trait anxiety along a continuum across the sample as a whole (whole-brain peak in the dorsal medial prefrontal cortex x,y,z=14,–8,52, $t$=5·09, p[FWE–cluster level]=0·0002; dorsal ROI p[FWE–cluster level]=0·0023; rostral ROI p[uncorrected]=0·001)), but crucially—since this is confounded by group effects—saw a correlation with trait anxiety in the patient group alone (dorsal peak x,y,z=–8,10,30, $t$=4·6, p[uncorrected]<0·0001) (figure 3A). Moreover, we recorded no correlations with depression ratings (on the BDI) across the whole group or within the patients with anxiety disorders alone (all p>0·2), which indicates that this effect was specific to anxiety symptoms.

Event-related analysis confirmed that the amygdala and the dorsal medial prefrontal cortex were significantly active in both patients with anxiety disorders and healthy controls across all participants (all trials vs baseline within ROIs: rostral x,y,z=–2,2,–50; $t$=4·01, p[FWE–voxel]=0·013; dorsal x,y,z=2,8,56, $t$=3·88; p[uncorrected]=0·0002; amygdala

x,y,z=28,–2,12, $t$=5·09, p[FWE–voxel]<0·0001). However, when we broke this analysis down into groups (ie, healthy participants and those with anxiety disorders), no significant interaction with groups was noted (rostral: diagnosis-by-valence x,y,z=–2,4,30, t=1·7, p[uncorrected]=0·045; dorsal: x,y,z=–2,2,28, $t$=1·9, p[uncorrected]=0·029; amygdala valence-by-diagnosis p>0·4). Exploratory whole-brain event-related activation interactions are presented in the appendix.

SPSS general linear models of estimates extracted from the a priori peak voxel showed no significant differences on variables of interest across DSM subdiagnoses (valence [fear, happy] by diagnosis [ie, generalised anxiety disorder, social anxiety disorder, both generalised and social anxiety disorder, or healthy] interaction: $F$[3,41]=2·2, p=0·1, partial eta² 0·1 [95% CI 0·00–0·29]; main effect of diagnosis p<0·2).

Restriction of the primary analyses to a primary diagnosis of generalised anxiety disorder (ie, including all patients with generalised anxiety disorder and those with comorbid generalised anxiety disorder and social anxiety disorder [n=15] but excluding those with social anxiety disorder only) replicated the whole-group effects (dorsal peak $vs$ trait anxiety: x,y,z=4,–8,38, $t$=3·88, p[uncorrected]<0·001; 1-tailed test of x,y,z=2,2,40 $vs$ trait anxiety: $r$[38]=0·3, p=0·04]). Thus, traditional subdiagnoses are not sufficient to explain the neurobiological abnormality in patients with anxiety disorders.

Task accuracy (correct identification of face emotion) was 81% across the whole study population, with a mean reaction time of 705 ms (SD 72) for happy faces and 708 ms (SD 71) for fearful faces across both groups. Valence had no significant effect on reaction time across the sample overall (fear $vs$ happy reaction time t[44]=0·4, p=0·7) or on a group-by-valence interaction in reaction time (p=0·14). However, in support of a brain–behaviour association, whole-brain analysis of all participants showed a peak in the dorsal anterior cingulate cortex driven by a significant negative association between fear and happy connectivity and fear versus happy reaction time (x,y,z=0,12,22; $t$=4; p[uncorrected]=0·0001). In other words, greater connectivity during the processing of fearful faces than happy faces was associated with a faster response to fearful faces than to happy faces.

## Discussion

This study confirmed our hypothesis that engagement of the aversive amplification circuit—recruited during stress in healthy individuals in our previous studies[7,9]—would be increased in the absence of shock threat in the context of pathological anxiety. Specifically, we show increased positive coupling within the dorsal medial prefrontal (anterior cingulate) cortex–amygdala circuit during fearful face processing in patients with generalised and social anxiety disorders. Moreover, we show that this increased coupling follows a continuum of trait anxiety, with the patients showing greatest coupling also presenting with the most severe
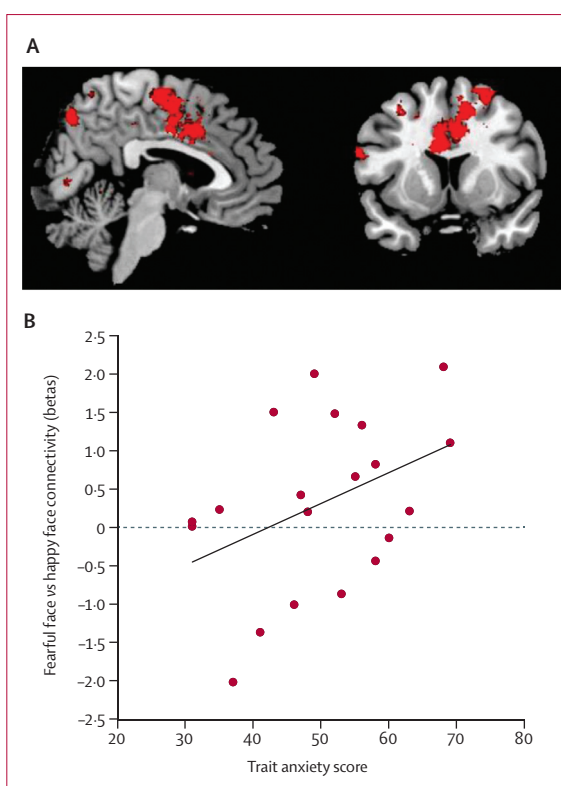
**Figure 3: Continuous variable analysis**
(A) Group map of the regions in the brain that correlate with trait anxiety for the fear $vs$ happy contrast. (B) Whole-brain positive correlation with trait anxiety scores: dorsal medial prefrontal cortex–amygdala coupling falls along a continuum of self-reported anxiety symptoms within the patient group. Data extracted from a priori peak (x,y,z=2,2,40) in the patient group for illustrative purposes.

symptoms. This effect overlaps the peaks emphasised in our previous study,[7] thus, we show the existence of a circuit that contributes to both adaptive anxiety responses and, when chronically activated, to maladaptive responses—a prerequisite for a more mechanistic, neurobiologically rooted diagnosis and treatment of pathological anxiety (panel).

We have previously called this circuit an aversive amplification circuit in accordance with findings from studies in rodents. These have shown the prelimbic prefrontal cortex to drive amygdala activity and lead to increased responses to fear.[10] In people, dorsal regions of the prefrontal cortex and anterior cingulate cortex have been argued to represent the human functional homologues of this region.[30,31] Previous work across several anxiety disorders has, for example, confirmed hyperactivity in the dorsal medial prefrontal cortex or dorsal anterior cingulate cortex or the amygdala in simple event-related studies.[5,18,19,32,33] Indeed, the studies by Milad and colleagues[30] and Mechias and colleagues[19] show a similar pattern to both our current and previous findings[7] of a larger more posterior cluster and a smaller more rostral prefrontal cluster. In these studies, the function of

these regions is argued to be in fear conditioning and conscious appraisal of threats, respectively—both of which align well with our proposed circuit function. The present study employs connectivity analysis to study the coupling between this dorsal region and the amygdala in a circuit in pathological anxiety. This approach is important because coupling is thought to represent a distinct informational process relative to activation,

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**Panel: Research in context**

**Systematic review**

This study was part of a programmatic sequence of studies and takes its main inspiration from these previous works. However, inspiration was also drawn from translational animal work[10,30,31] and both original trials and review papers exploring activity within the studied brain regions in both patients and healthy populations.[2–5,17–19,32,33] Further inspiration was drawn from data (again from both original studies and reviews) exploring connectivity within this circuit[13] and a related ventral circuit[11–15] in both patients and healthy controls. Relevant articles were identified through searches of PubMed and Google Scholar including combinations of the terms "anxiety", "stress", "anxiety disorders", "GAD", "social anxiety", "PPI", "fMRI", "connectivity", "amygdala","coupling", "dACC","dmPFC" in August, 2013. Our search was of the entire PubMed catalogue (from start until present) and we included articles published in English only. To the best of our knowledge, this study is the first to explore connectivity within this circuit across social or generalised anxiety disorder and healthy controls.

**Interpretation**

The present data, together with our previous work, suggest that a common mechanism, namely positive dorsal medial prefrontal cortex–amygdala coupling during aversive processing, might underlie both healthy stress responses and social and generalised anxiety disorders. Moreover, this mechanism might track subjective anxiety symptoms such that increased recruitment of this circuit is associated with greater self-reported trait anxiety. Although this report is early experimental work, the findings have two potential clinical implications. First, this study is a step away from categorical diagnoses based on symptoms and towards a more continuum-based, mechanistic understanding of anxiety pathology. Specifically, the data provide experimental support for the idea that anxiety subtypes might share overlapping neurobiological abnormalities that fall along a continuous scale from adaptive to pathological. Second, from a clinical perspective, these data might ultimately help to target treatments. We have shown this circuit to be modulated by serotonin and as such we provide a potential mechanism by which such drugs enact their anxiolytic properties. This could, in turn, provide a potential means of identifying individuals who will respond to such treatments (eg, patients who would be better suited to psychological or pharmacological interventions).

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which specifically represents the flow of information and attentional processes.[20] It is not that these regions are any more or less efficient at processing information in the pathological disorder; rather, the extent to which they communicate is changed.

Our findings therefore allow us to map a potential neural pathway for a key symptom that unites anxiety disorders, namely chronically increased threat processing. The amygdala might detect threats, but the dorsal medial prefrontal (anterior cingulate) cortex could be a central node of a broader anxiety circuit, playing a key part in integration of threat information and orchestration of response expression through synchronised activity with distant brain regions. Thus, this circuit is activated in stressful environments (eg, shock anticipation[7]) to promote the adaptive[34] detection of threatening stimuli (at the expense of non-threatening stimuli [Vytal K, unpublished]). In healthy people under innocuous circumstances, mild threats (eg, fearful faces) do not increase dorsal medial prefrontal cortex–amygdala coupling, but in pathological anxiety this circuit becomes permanently switched on, even in innocuous contexts, and contributes to a crippling focus upon negative life experiences. Of course, this is not the only symptom characteristic of pathological anxiety, but it is a core feature that perhaps unifies both adaptive and pathological anxiety.

Our ability to map a potential symptom pathway from adaptive to maladaptive states supports the clinical potential of this circuit. First, from a diagnostic perspective, we show that neural circuit engagement exists along a continuum as a function of self-reported symptoms, and irrespective of the traditional DSM-defined diagnosis of generalised or social anxiety disorder. Psychiatry is the only branch of medicine in which diagnosis is predominantly based upon self-reported symptoms rather than underlying mechanisms; the present findings provide the potential beginnings of a dimensional, mechanistic anxiety index with diagnostic value.[21,35] Anxiety disorder subtypes are highly comorbid[36,37] and our present data are consistent with the assumption that this is because, at least as far as generalised and social anxiety disorders go, the neural circuit underlying a core symptom of anxiety disorders—a bias towards threats—falls along a diagnosis-independent continuum. Such a scale could comprise a row of the "negative valence symptoms" category of the Research Domains Criteria matrix[35,38] which seeks to create biologically informed psychiatric diagnoses. Future work with a much larger sample of individuals, a broader range of anxious traits, and undertaken across several sites will be the next step towards translation of this finding into a clinically useful measure.

Second, our previous work with this circuit provides a mechanism by which we might be able to target treatments. The direction of correlation between symptom severity and circuit engagement suggests that we should attempt to disrupt activity within this circuit. We have, in fact, shown this circuit to be inhibited by

serotonin,[8] such that serotonin reduction serves to increase activity within this circuit during the processing of fearful faces. Thus, selective serotonin reuptake inhibitors could restore inhibition of this circuit,[39] reducing responses to aversive stimuli. Such an understanding is key because, despite the widespread use of selective serotonin reuptake inhibitor drugs, our understanding of their mechanism of action is far from adequate,[39] leading to inefficient prescription. Perhaps this circuit will provide a means of recognising patients who will respond positively to treatment. In a specialty in which a large proportion of patients do not respond to their first treatment, even a small increase in success rate would be beneficial.

Future work can therefore ask: what interventions—pharmacological or psychological—can serve to attenuate activity within this circuit during aversive processing? Evidence suggests, for example, that cognitive-based treatments can target nodes within this circuit.[40] One of the biggest impediments to treatment development is the failure of animal screens to scale up to human beings and a lack of naturalistic human screening markers.[1,41] Since we have shown that this circuit can be safely and reversibly activated in healthy individuals,[8] while simultaneously linking it to clinical presentation, we might be able to use provocation of this circuit as a screen for more targeted assessment of candidate anxiolytics.[1,42]

To show the validity of our findings from several angles, we obtained traditional subtype diagnoses. This analysis replicated key effects independent of subdiagnosis, but it should be noted that our naturalistic sample contained fewer people with a diagnosis of social anxiety disorder alone. Additional analyses suggested that this did not unduly affect the overall findings, but extra caution should be exercised in drawing conclusions about this particular subgroup because of the sample size.

Moreover, and importantly, we are not arguing that this is the sole role of this circuit and, furthermore, we are somewhat agnostic regarding naming the prefrontal region (eg, dorsal medial prefrontal cortex vs dorsal anterior cingulate cortex). What is crucial is that we have noted an overlapping pattern across several studies examining anxiety-related processes using matched tasks.

In conclusion, this study shows engagement of the dorsal medial prefrontal (anterior cingulate) cortex–amygdala circuit during aversive processing in pathological anxiety. A detailed understanding of the relation between neural circuitry and such core anxiety symptoms is, we argue, a prerequisite for more targeted diagnosis and treatments. We hope that this study represents a first step towards a more mechanistic and dimensional understanding of the pathology underlying anxiety disorders.

**Contributors**
OJR and CG designed the trial; LL and PA recruited and scheduled participants; OJR, LL, KV, and PA tested the participants; OJR, LL, and PA processed the data; OJR and CG analysed the data; and CG, OJR, and KV wrote the report.

**References**
1 Robinson OJ, Vytal K, Cornwell BR, Grillon C. The impact of anxiety upon cognition: perspectives from human threat of shock studies. *Front Hum Neurosci* 2013; **7:** 203.
2 Cools R, Calder A, Lawrence A, Clark L, Bullmore E, Robbins T. Individual differences in threat sensitivity predict serotonergic modulation of amygdala response to fearful faces. *Psychopharmacology* 2005; **180:** 670–79.
3 Harmer CJ, Mackay CE, Reid CB, Cowen PJ, Goodwin GM. Antidepressant drug treatment modifies the neural processing of nonconscious threat cues. *Biol Psychiatry* 2006; **59:** 816–20.
4 Roozendaal B, McEwen BS, Chattarji S. Stress, memory and the amygdala. *Nat Rev Neurosci* 2009; **10:** 423–33.
5 Etkin A. Functional neuroanatomy of anxiety: a neural circuit perspective. *Curr Top Behav Neurosci* 2010; **2:** 251–77.
6 Price JL, Drevets WC. Neural circuits underlying the pathophysiology of mood disorders. *Trends Cogn Sci* 2012; **16:** 61–71.
7 Robinson OJ, Charney DR, Overstreet C, Vytal K, Grillon C. The adaptive threat bias in anxiety: amygdala–dorsomedial prefrontal cortex coupling and aversive amplification. *Neuroimage* 2012; **60:** 523–29.
8 Robinson OJ, Overstreet C, Allen PS, et al. The role of serotonin in the neurocircuitry of negative affective bias: serotonergic modulation of the dorsal medial prefrontal-amygdala 'aversive amplification' circuit. *Neuroimage* 2013; **78:** 217–23.
9 Vytal KE, Overstreet C, Charney DR, Robinson OJ, Grillon C. Sustained anxiety increases amygdala-dorsomedial prefrontal coupling: a mechanism for maintaining an anxious state in healthy adults. *J Psychiatry Neurosci* 2014; **39:** 130145.
10 Sierra-Mercado D, Padilla-Coreano N, Quirk GJ. Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. *Neuropsychopharmacology* 2011; **36:** 529–38.
11 Pezawas L, Meyer-Lindenberg A, Drabant EM, et al. 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. *Nat Neurosci* 2005; **8:** 828–34.
12 Kim MJ, Loucks RA, Palmer AL, et al. The structural and functional connectivity of the amygdala: from normal emotion to pathological anxiety. *Behav Brain Res* 2011; **223:** 403–10.
13 Etkin A, Egner T, Kalisch R. Emotional processing in anterior cingulate and medial prefrontal cortex. *Trends Cogn Sci* 2011; **15:** 85–93.
14 Passamonti L, Crockett MJ, Apergis-Schoute AM, et al. Effects of acute tryptophan depletion on prefrontal-amygdala connectivity while viewing facial signals of aggression. *Biol Psychiatry* 2012; **71:** 36–43.
15 Motzkin JC, Philippi CL, Wolf RC, Baskaya MK, Koenigs M. Ventromedial prefrontal cortex is critical for the regulation of amygdala activity in humans. *Biol Psychiatry* 2014; published online Feb 26. DOI:10.1016/j.biopsych.2014.02.014.
16 Robinson OJ, Letkiewicz AM, Overstreet C, Ernst M, Grillon C. The effect of induced anxiety on cognition: threat of shock enhances aversive processing in healthy individuals. *Cogn Affect Behav Neurosci* 2011; **11:** 217–27.
17 Rauch SL, Shin LM, Wright CI. Neuroimaging studies of amygdala function in anxiety disorders. *Ann NY Acad Sci* 2003; **985:** 389–410.
18 Shin LM, Wright CI, Cannistraro PA, et al. A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. *Arch Gen Psychiatry* 2005; **62:** 273–81.

19 Mechias M-L, Etkin A, Kalisch R. A meta-analysis of instructed fear studies: implications for conscious appraisal of threat. *Neuroimage* 2010; **49:** 1760–68.

20 Salinas E, Sejnowski TJ. Correlated neuronal activity and the flow of neural information. *Nat Rev Neurosci* 2001; **2:** 539–50.

21 Kupfer DJ, Regier DA. Neuroscience, clinical evidence, and the future of psychiatric classification in DSM-5. *Am J Psychiatry* 2011; **168:** 672–74.

22 First MB, Spitzer RL, Gibbon M, Williams JBW. Structured clinical interview for DSM-IV-TR axis I disorders—patient edition (SCID-I/P, 11/2002 revision). New York: New York State Psychiatric Institute, 2002.

23 Spielberger CD, Gorsuch RL, Lushene RE. The state-trait anxiety inventory. Palo Alto: Consulting Psychologists Press Inc, 1970.

24 Beck AT, Steer RA. Beck depression inventory: manual. New York: Psychological Corporation, 1987.

25 Wechsler D. Wechsler abbreviated scale of intelligence. New York: Psychological Corporation, 1999.

26 Tzourio-Mazoyer N, Landeau B, Papathanassiou D, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 2002; **15:** 273–89.

27 Brett M, Anton JL, Valabregue R, Poline JB. Region of interest analysis using an SPM toolbox. *Neuroimage* 2002; **16:** abstr 497.

28 Brett M, Johnsrude IS, Owen AM. The problem of functional localization in the human brain. *Nat Rev Neurosci* 2002; **3:** 243–49.

29 Hoaglin DC, Iglewicz B. Fine-tuning some resistant rules for outlier labeling. *J Am Stat Assoc* 1987; **82:** 1147–49.

30 Milad MR, Quirk GJ, Pitman RK, Orr SP, Fischl B, Rauch SL. A role for the human dorsal anterior cingulate cortex in fear expression. *Biol Psychiatry* 2007; **62:** 1191–94.

31 Quirk GJ, Beer JS. Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. *Curr Opin Neurobiol* 2006; **16:** 723–27.

32 Graham BM, Milad MR. The study of fear extinction: implications for anxiety disorders. *Am J Psychiatry* 2011; **168:** 1255–65.

33 Shin LM, Bush G, Milad MR, et al. Exaggerated activation of dorsal anterior cingulate cortex during cognitive interference: a monozygotic twin study of posttraumatic stress disorder. *Am J Psychiatry* 2011; **168:** 979–85.

34 Grupe DW, Nitschke JB. Uncertainty and anticipation in anxiety: an integrated neurobiological and psychological perspective. *Nat Rev Neurosci* 2013; **14:** 488–501.

35 Insel T, Cuthbert B, Garvey M, et al. Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. *Am J Psychiatry* 2010; **167:** 748–51.

36 Kessler RC, Avenevoli S, McLaughlin KA, et al. Lifetime co-morbidity of DSM-IV disorders in the US National Comorbidity Survey Replication Adolescent Supplement (NCS-A). *Psychol Med* 2012; **42:** 1997–2010.

37 Goisman RM, Goldenberg I, Vasile RG, Keller MB. Comorbidity of anxiety disorders in a multicenter anxiety study. *Comp Psychiatry* 1995; **36:** 303–11.

38 Cuthbert B, Insel T. Toward the future of psychiatric diagnosis: the seven pillars of RDoC. *BMC Med* 2013; **11:** 126.

39 McCabe C, Mishor Z, Filippini N, Cowen PJ, Taylor MJ, Harmer CJ. SSRI administration reduces resting state functional connectivity in dorso-medial prefrontal cortex. *Mol Psychiatry* 2011; **16:** 592–94.

40 Straube T, Glauer M, Dilger S, Mentzel H-J, Miltner WH. Effects of cognitive-behavioral therapy on brain activation in specific phobia. *Neuroimage* 2006; **29:** 125–35.

41 Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. *Nat Neurosci* 2010; **13:** 1161–69.

42 Harmer CJ, Cowen PJ, Goodwin GM. Efficacy markers in depression. *J Psychopharmacol* 2011; **25:** 1148–58.