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Anxiety vulnerability is associated with altered anterior cingulate response to an affective appraisal task

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Abstract

The anterior cingulate cortex (ACC) is critically involved not only in affective and anxiety processing, but also in error and conflict monitoring. To investigate how anxiety interacts with processing affective ambiguity, 15 anxious and 15 nonanxious individuals were scanned while performing a validated affective appraisal task, in which the fraction of faces of a particular affect or gender was parametrically controlled to provide various levels of ambiguity. The anxious group showed less ventral and greater dorsal ACC activation during ambiguous affective relative to ambiguous gender stimuli. For anxious individuals, dorsal ACC activation was related to a more biased response. Collectively, these data indicate that anxious individuals activate the dorsal and ventral components of the ACC differently during affective appraisal.

Keywords

anterior cingulate; anxiety; face processing; functional magnetic resonance imaging

Introduction

Anxiety disorders are the most common type of mental illness in the United States [1], and negatively impact quality of life [2]. One approach to understanding the phenomenology of anxiety is to compare the responses of young anxiety prone adults, i.e. individuals with an anxious temperament, with those of individuals with normal levels of trait anxiety.

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Supplementary data

Supplementary data available online at *NeuroReport* (www.neuroreport.com).

Earlier investigations reveal that anxious individuals [3] show altered biases when attempting to disambiguate affective information. Related studies have specifically implicated the ventral anterior cingulate cortex (ACC) in affective processing [4,5]. This structure may be particularly involved in processing affective information when attention is focused internally, i.e. on the self [6,7]. Conversely, there is evidence that the dorsal ACC is activated when attention is focused externally, and when the cognitive control mechanism is engaged [8,9]. Recent imaging studies suggest that the dorsal ACC (BA 24 and 32) [5] and medial prefrontal cortex (MPFC) (including BA 8, 6) are used for processing externally relevant information and the ventral areas of the ACC (BA 24, 25, 32, and 33) and MPFC (including BA 10 and ventral ACC) are used for processing more internally focused information [10]. This differentiation is consistent with evidence indicating that the ventral ACC is activated less in anxious traumatized patients versus controls in the differential activation between affective and nonaffective tasks [11], potentially because the anxious patients were more chronically anxious and worried during all phases of the task.

Ambiguity processing occurs when an individual makes a decision based on information that does not clearly suggest the selection of one option over another. Ambiguity can arise because the information is incomplete, contradictory, or unclear. Altered levels of ambiguity have been hypothesized to play a critical role in anxiety disorders [12]. Both behavioral and functional neuroimaging studies have shown that individuals with various anxiety disorders perform differently than do individuals without anxiety disorders [12]. In an earlier study [13], we devised a wall of faces task to examine the neural substrates underlying emotional ambiguity and found that healthy volunteers exhibited: (i) an increased activation in the ventral ACC when processing stimuli that contained groups of affective faces with no predominant affect (i.e. ambiguous affective stimuli) relative to stimuli that contained groups of faces with no predominant gender (i.e. ambiguous gender stimuli) and (ii) an increased activation in the dorsal ACC related to processing all ambiguous versus unambiguous sets (i.e. both gender and affective sets) [13]. The activation pattern observed when individuals determined the predominant affect of an ambiguous group of faces is very similar to the activation seen in other ambiguous facial expression tasks, such as ambiguous, morphed, or composite faces [14]. Although this form of ambiguous processing activates the ventral ACC in controls, based on earlier research, highly anxious individuals may not be able to differentially disengage this region. Based on these observations, we predicted that performance of an ambiguity-processing task would be associated with decreased ventral ACC activity and increased dorsal ACC activity in anxiety prone relative to anxiety normative individuals.

Methods and materials

Participants

All participants provided written informed consent to participate in this study, which was approved by the University of California San Diego and San Diego State University Institutional Review Boards. Initially, undergraduate San Diego State students participated in screening using the Spielberger Trait Anxiety Questionnaire [15]. Subsequently, a group of individuals who scored high in trait anxiety (in the upper 15th percentile of the distribution)

and a second group who had normative levels of trait anxiety (from the 40th–60th percentile of the distribution) were selected for further screening. Thirty participants were studied, see Table 1. All participants underwent the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorder-IV [16] to determine the presence of current and lifetime psychiatric illness, see Table 1. None of the participants had ever sought treatment for their anxiety symptoms or taken psychotropic medications in the earlier 12 months. Both groups were recruited from the same university, and did not differ significantly with regard to their year in school. All participants were trained to perform the affective appraisal task before testing during functional magnetic resonance imaging scanning (fMRI), and were paid to participate in the study. All participants completed a multifactor personality measure, Neuroticism Extraversion Openness-Personality Inventory-Revised (NEO-PIR) [17]; T-scores were calculated and are reported for neuroticism, extroversion, and openness to new experience.

Procedure

All participants performed the affective appraisal task during fMRI. For a full description, see Simmons *et al.* 2006 [13]. The key aspects of the wall of faces task are as follows: 32 faces are presented that vary in the ratio of angry/happy and male/female faces. Participants answer if there are more ‘angry or happy’ faces in the affective trials or more ‘female or male’ faces in the gender trials. Affective and gender trials were equally split between ambiguous (16 of each face type) and nonambiguous (6/26). Affective and gender trials were psychometrically matched so that all facial characteristics are equally present across conditions. The response bias (i.e. how many faces were required for the participant to determine that the group was angry more than 50% of the time) was calculated by interpolating the slope of the response selections. The lower the response bias, the fewer faces were required to classify the array as angry.

Functional magnetic resonance imaging analysis pathway

During the task, an fMRI run sensitive to blood oxygenation level-dependent contrast was collected for each participant using a 1.5 T Siemens scanner (Siemens, Munich, Germany) (T2* weighted echo planar imaging; repetition time (TR)=2000 ms, echo time (TE)=40ms, 64×64 matrix, 204-mm axial slices, 256 scans). fMRI acquisitions were time locked to the onset of the task. During the same experimental session, a high-resolution, T1-weighted image (MPRAGE, Siemens; TR=11.4ms, TE=4.4ms, flip angle=10°, field of view (FOV)= 256×256 , 1 mm³ voxels) was obtained for anatomical reference.

The data were preprocessed and analyzed with the software AFNI (National Institute of Mental Health, Bethesda, Maryland, USA) [18]. Data were normalized to Talairach coordinates [19]. The echoplanar images were realigned to a central slice selected to minimize differentially acquired scans and time corrected for slice acquisition order. To exclude the voxels showing an artifact-related signal drop, a combined threshold/cluster-growing algorithm was applied. The preprocessed time series data for each individual were analyzed using a multiple regression model consisting of 11 regressors. There were six task-related regressors, which identified the time series for the three ratios (6/26, 16/16, and 26/6) of gender (female–male) and emotion (angry–happy). Each regressor was created using a

reference function corresponding to the 3 s during a trial during which participants viewed the array of faces. These regressors were convolved with a prototypical hemodynamic response function before inclusion in the regression model. In addition, three regressors were used to account for residual motion (in the roll, pitch, and yaw direction), and a baseline regressor and linear trends regressor were used to eliminate slow signal drifts. The AFNI program, 3dDeconvolve, was used to calculate the estimated voxel-wise response amplitude. A 6 mm full-width half-maximum Gaussian filter was applied to the voxel-wise percent signal change data to account for individual variations of anatomical landmarks.

Statistical analyses

Our first analysis tested for differential BOLD signal between groups in contrast to ambiguous gender and ambiguous affective trials. The second analysis tested differential activation between groups in a conjunction analysis, i.e. each voxel had to satisfy simultaneously several threshold criteria. In particular, we examined voxels that showed significant activation during ambiguous relative to unambiguous trials during both affective and gender conditions. Voxel-wise percent signal change data were entered into a series of T-tests, one for each contrast (ambiguous gender versus ambiguous affect; the conjunction of ambiguous gender and ambiguous affect). A threshold adjustment method based on Monte-Carlo simulations was used to guard against identifying false-positive areas of activation. Based on these simulations, it was determined that a voxel-wise *a priori* probability of 0.05 would result in a corrected cluster-wise activation probability of 0.05 if a minimum volume of 1024 μ l and a connectivity radius of 4.0 mm was considered. For the conjunction analysis, the minimum volume was lowered to 128 μ l as multiple comparisons were performed. Finally, the average percent signal difference was extracted from regions of activation that survived this threshold/cluster method.

All analyses for the behavioral data were carried out with SPSS 12.0 (SPSS Inc., Chicago, Illinois, USA). Simple *t*-tests were used to measure the task effects and a mixed model analysis of variance (fixed factor: task conditions; random factor: participants) was used to analyze group differences in the behavioral measures. (see Supplementary Materials for results).

Pearson's product correlations were calculated for the functional data with behavioral measures and the first three factors (neuroticism, extroversion, and openness) of the NEO-PIR.

Results

Functional neuroimaging

The anxiety prone group showed significantly greater activation than the anxiety normative group in bilateral dorsal ACC, bilateral middle temporal gyrus, bilateral superior frontal gyrus, right inferior frontal gyrus, and left posterior insula for ambiguous affective trials minus ambiguous gender trials. In comparison, the anxiety normative group showed significantly greater activation than the anxiety prone group in the ventral ACC (see Table 2; Fig. 1 for this contrast).

Correlational

To determine whether the degree of effort was related to activation differences in the ACC, we conducted a correlational analysis between the amount of task-related brain activation and latency to respond to all (i.e. affective and gender) trials in the dorsal ACC (Spearman's $\rho=0.018$, $P=0.927$ and Spearman's $\rho=0.086$, $P=0.65$, respectively) or ventral ACC (Spearman's $\rho=0.115$, $P=0.542$ and Spearman's $\rho=0.131$, $P=0.488$, respectively). However, in the anxiety prone (but not in the anxiety normative) group, a significant positive correlation was observed between response selection bias (as described above) and dorsal ACC activation produced by the between groups contrast of ambiguous affective trials minus ambiguous gender trials (see Fig. 2). Personality measures (i.e. NEO-PIR domain scores) did not correlate significantly with differential regional brain activity within the anxiety prone group for neuroticism (Spearman's $\rho=0.399$, $P=0.142$ and Spearman's $\rho=0.067$, $P=0.812$, for dorsal and ventral ACC, respectively), extroversion (Spearman's $\rho=0.305$, $P=0.270$ and Spearman's $\rho=-0.194$, $P=0.486$, for dorsal and ventral ACC, respectively), and openness (Spearman's $\rho=0.259$, $P=0.350$ and Spearman's $\rho=0.082$, $P=0.773$, for dorsal and ventral ACC, respectively).

Discussion

This investigation yielded two main findings. First, the anxiety prone group relative to the anxiety normative group showed less ventral ACC activation and greater dorsal ACC activation when processing ambiguous affective versus ambiguous gender trials (Table 2). Second, within the anxiety prone group, dorsal ACC activation for this contrast correlated significantly with response bias (i.e. the point at which the individual assesses the faces as angry half the time and happy half the time), such that the greater the activation in the dorsal ACC, the fewer the number of angry faces that were required for the group to be considered angry (Fig. 2). These findings should be considered in the context of a growing body of evidence of functional subdivisions within ACC, in which the ventral region handles emotional information and the dorsal region deals with cognitive decisions [5]. In related literature, the ventral regions of the ACC relate to processing self-relevant information [20], whereas dorsal ACC activation relates to processing information that requires sustained externally focused attention [10,21]. Based on earlier work, we suggest that demanding cognitive and emotional processing activates the ventral ACC when attention is directed internally and the dorsal ACC when attention is directed externally. In the current study, the observation that anxiety prone individuals are using dorsal ACC more may suggest that ventral ACC did not contribute to disambiguation in this group, potentially owing to greater and less variable anxiety (or selfrelevant processing) across all conditions [22].

The finding in anxiety prone participants that increased dorsal ACC activation is correlated with a negative response bias is consistent with this model if one assumes that the lack of a positive response bias in the anxiety prone participants was owing to a greater vigilance toward an external stimulus during ambiguity. Specifically, we suggest that, when making affective judgments in the presence of substantial uncertainty (ambiguity), anxious individuals become more vigilant to negative external bias, and thus may show greater dorsal MPFC/ACC responses.

In the current study, one potential clue into how anxiety affects determination of ambiguous stimuli comes from the correlation analysis. In the anxiety prone group, increased sensitivity (as measured by response bias) to angry faces was associated with an increased activity in the dorsal ACC. This may suggest that the response bias in the anxiety prone group is owing to an increased utilization of the dACC resulting in a more negative bias. Future studies should investigate whether individuals who show greater dorsal activation to affective decision-making are more vulnerable to the development of clinical mood and anxiety disorders, and if the activation is responsive to psychological or psychopharmacological treatment.

Increased activation for the ambiguous affective versus ambiguous gender trials in the anxiety prone group was also found in the bilateral middle temporal gyrus, bilateral superior frontal gyrus, right inferior frontal gyrus, and left posterior insula; these are all regions that have been previously shown to be part of a neural circuit involved in affective processing, particularly in anxious participants [23]. Anatomical studies on the ACC have found strong neural connections with the insula, superior, and inferior frontal gyrus [24]. The differential group activation for this contrast in these areas may be indicative of a neural network that is involved in anxiety processing [6].

This study has several limitations. First, the ambiguous stimuli were also more difficult (as reflected in the increased response latencies). Therefore, any subtraction of ambiguous and unambiguous stimuli is confounded by task difficulty, with the exception of the main contrast of interest for this study, i.e. affective versus gender ambiguity conditions, as these had similar response latencies. Second, there was task switching, and this switching likely did recruit brain regions important for executive functioning. A third concern is that individuals may have realized that some sets of faces had equal numbers of male and female (or happy and angry) faces. Therefore, for these individuals, this condition was not ambiguous. A fourth concern is that the lack of anxiety prone individuals without psychiatric conditions may have obscured whether the observed effects are dependent on increased anxiety proneness or actual clinical diagnosis. Finally, it should be noted that although there was no significant difference in amygdala activation between groups, this is not unexpected as affective and gender judgments of the type used in this study have not produced differential activation in the amygdala in earlier research [25], most likely owing to being parametrically matched for the ratio of happy and sad faces between contrasted conditions.

Conclusion

The results of the current study are consistent with the hypothesis that anxious individuals engage subregions of the ACC differently than do nonanxious individuals when attempting to disambiguate an emotionally ambiguous stimulus. In particular, anxiety prone individuals activate the dorsal ACC more (and the ventral ACC less) during decision-making in the context of affective ambiguity, and the amount of activation in the dorsal ACC relates to the bias in the capacity to detect angry faces. It remains to be determined to what extent these differences in the neural substrates subserving affective processing etiologically contribute to or, merely, reflect a vulnerability to anxiety disorders. To answer this question, these

results will need to be replicated in larger samples, and extended to other affective decision-making tasks as well as additional at-risk and anxiety-disordered populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Dorsal (D) and ventral (V) medial prefrontal cortex (MPFC) differential activation for type of ambiguity (i.e. affective vs. gender) in anxiety prone versus anxiety normative groups (anxiety prone > anxiety normative and anxiety prone < anxiety normative are shown in dark grey and light grey, respectively). AN, anxiety normative; AP, anxiety prone.

A scatter plot showing the relationship between Response bias (X-axis, ranging from 12 to 18) and Percent signal change in the DACC (Y-axis, ranging from -0.80 to 0.20). The plot includes two data series: Anxiety prone (open circles) and Anxiety normative (filled circles). Both series show a negative correlation, with a regression line fitted to each. The Anxiety normative group generally shows higher signal change values compared to the Anxiety prone group across the range of response bias.

Table 1

Demographic and diagnostic variables for AN and AP groups

Variable	Anxiety normative	Anxiety prone
Age	18.9 (1.4)	18.7 (0.7)
Education	13.4 (0.7)	13.5 (0.8)
Gender		
Female	12	11
Male	3	4
Diagnosis		
No diagnosis	15	7 ^a
GAD only	0	4
GAD+SAD	0	3
GAD + SAD + PD	0	1
GAD + SAD + PD + OCD	0	1

Data presented as mean (standard deviation) were appropriate. Groups did not differ significantly on demographic variables.

GAD, generalized anxiety disorder; OCD, obsessive compulsive disorder; PD, panic disorder; SAD, social anxiety disorder

^aSix had subthreshold GAD and/or SAD.

Areas of activation for affective ambiguousminus gender ambiguous trials that differed between the AP and AN groups

Table 2

Volume	x	y	z	Side	Location	BA	
AP> AN							
1920	45	-30	10	R	Transverse temporal gyrus	41	
1856	-1	49	42	R/L	Superior frontal gyrus	8	
1408	50	11	12	R	Inferior frontal gyrus	44	
1408	-48	-59	22	L	Middle temporal gyrus	39	
1408	-4	32	23	R/L	Dorsal anterior cingulate	32	
1088	-41	-25	20	L	Posterior insula	13	
AN > AP	1280	6	35	-5	R	Ventral anterior cingulate	32

BA, Brodmann Area; L, left; R, right.