

Fear-related activity in subgenual anterior cingulate differs between men and women

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Functional magnetic resonance imaging in association with an instructed fear/anticipatory anxiety paradigm was used to explore sex differences in the human fear response. During anticipation of mild electrodermal stimulation, women, as compared with men, demonstrated increased activity in the subgenual anterior cingulate cortex and functionally related regions of the insula and brainstem. The subgenual anterior cingulate cortex is a region

critical for emotional control implicated in the pathogenesis of psychiatric disease. Present findings suggest a contributory neural substrate for the greater susceptibility of women to anxiety and affective disorders, and emphasize the importance of considering participant sex when designing and interpreting functional neuroimaging studies. *NeuroReport* 16:1233–1236 © 2005 Lippincott Williams & Wilkins.

Key words: Anticipatory anxiety; Emotion; Functional magnetic resonance imaging; Instructed fear; Sex; Sex differences; Ventromedial prefrontal cortex

INTRODUCTION

Human psychiatric disorders result from as yet poorly characterized interactions between environmental stressors and predisposing factors – including sex – and have been associated with alterations in frontal and limbic brain regions [1–5]. Functional neuroimaging has revealed sex differences in these regions at rest [6] and in response to negative emotional conditions [7–11]. Women are significantly more likely than men to suffer from affective and anxiety disorders [12]. Following a transient stressful experience, females – both human [13] and animal [14] – develop persistent emotional dysfunction at different rates than males, suggesting the existence of sex differences, which may be particularly relevant under conditions of stress, in frontal–limbic functioning. In laboratory animals, the fear-conditioning paradigm has been widely used to probe this circuitry, highlighting the role of the ventromedial prefrontal cortex (vmPFC) and amygdala [15]. The present study used functional magnetic resonance imaging and an instructed fear paradigm [16] modeled on animal fear-conditioning experiments to probe fear circuitry and explore the neurobiological basis for sex differences in the human fear response.

METHODS

This study was approved by the local Institutional Review Board. Twenty-three right-handed participants (10 women, mean age 27.4 years; 13 men, mean age 27.9 years) took part

in the study, all of whom were free from medication, substance abuse, medical, neurologic and psychiatric disease. Immediately before scanning, participants themselves determined the level of mild electrodermal stimulation delivered to their left wrist using a dial-up procedure, according to which stimulations were increased gradually in intensity to attain an ‘uncomfortable but not painful’ level of sensation. Participants were then told ‘all stimulations you receive during this study will be of exactly this strength and duration’. During an experimental condition of threat, participants were told, ‘an electrodermal stimulation can occur at any time’. During safety, participants knew they would receive no stimulations. Threat and safety were signified by the presentation of easily distinguishable blocks of colors via a magnetic resonance-compatible screen. Pairing of colors with conditions was counterbalanced across participants. Each color appeared for 12 s, followed by an 18 s rest period, with 10 blocks of each color per study session. Participants did not actually receive electrodermal stimulation during scanning.

Image acquisition: Gradient echoplanar functional images (TR=1200; TE=30; flip angle=70°; FOV=240 mm; fifteen 5 mm slices; 1 mm interslice space; matrix=64 × 64) sensitive to blood oxygen level-dependent signal were obtained axially on a GE-Sigma 3T magnetic resonance image scanner using a modified z-shimming algorithm to minimize susceptibility artifact at the base of the brain.

T1-weighted anatomic images were acquired using a spoiled gradient sequence with a resolution of $0.9375 \times 0.9375 \times 1 \text{ mm}^3$.

Image processing and analysis: Image processing within a customized Statistical Parametric Mapping (SPM) software package (Wellcome Department of Imaging Neuroscience, University College, London, UK) consisted of realignment, coregistration of functional data to each participant's anatomic image, stereotactic normalization and spatial smoothing. Examination of realignment parameters revealed no significant head movement, defined as greater than one-third of a voxel, in all but one participant. The affected portion of this participant's data was excluded from analysis. A multiple linear regression model at the participant level determined the extent to which each voxel's activity correlated with stimulus onset times convolved with a prototypical hemodynamic response function, resulting in a condition-specific contrast image for each participant, which was entered into a random-effects analysis to assess between-sex functional differences. Functional results were assessed at an initial threshold of $p < 0.005$, uncorrected, cluster volume > 3 voxels (81 mm^3). Predicted peaks in the frontal-limbic regions of a priori interest were further assessed using the small volume correction function (to the spatial extent of the nearest cluster, on the basis of coordinates for the amygdala derived from [16] and for the vmPFC/subgenual anterior cingulate cortex (sgAC) derived from [3]) and were considered significant if the corresponding voxelwise p value was less than 0.05, corrected for multiple comparisons. Anatomic images were analyzed using voxel-based morphometry performed according to standard SPM methods to determine whether structural differences contributed to functional findings.

RESULTS

Between-sex functional results are presented in Table 1 and Fig. 1. Greater threat-related activity was observed in women than in men, in the right sgAC, right insula and a region of the superior medial dorsal brainstem. Greater threat-related sgAC activity in women corresponded not only to an increase in comparison to men but also to an increase in comparison to safety and to a resting baseline. In

Table 1. Clusters of significantly different activation during threat between women and men.

Region	Coordinates			Volume (mm ³)	Z score	p value
	x	y	z			
<i>Increased in women as compared with men</i>						
Dorsal midbrain	6	-33	-6	837	3.08	0.001
Right insula (BA 13)	42	3	-3	81	2.84	0.002
Right sgAC (BA 24)	6	21	-6	162	2.83	0.002/0.007 (corr)
<i>Increased in men as compared with women</i>						
Left postcentral gyrus	-51	-21	36	1377	3.28	0.001
Right sup. frontal gyrus	21	69	6	216	2.84	0.002

Coordinates are in MNI space. When appropriate, p values corrected for multiple comparisons on the basis of anatomically specified a priori hypotheses are reported alongside uncorrected p values.

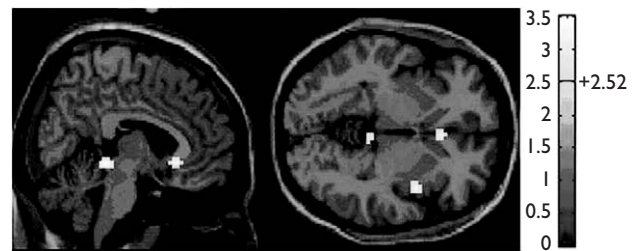


Fig. 1. Areas of increased neural activity in women as compared with men during threat. Blood oxygen level-dependent (BOLD) functional magnetic resonance image activations are displayed as a T-map at a $p < 0.01$ (uncorrected) threshold, overlaid onto canonical T1 sagittal and axial sections. Foci of significant activation are seen in the right sgAC, right insula and dorsal brainstem.

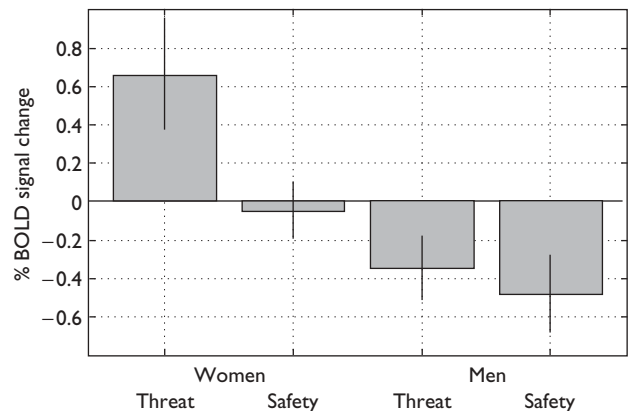


Fig. 2. Bar plot of sex-specific and condition-specific activity in the right subgenual anterior cingulate ($x=6, y=21, z=-6$) depicting increased activity in women during threat, and decreased activity in the same region in men during both threat and safety. Zero corresponds to a resting baseline. Vertical bars indicate standard error.

contrast, men demonstrated *decreased* sgAC activity during threat as compared with a resting baseline, as shown by examination of sex-specific and condition-specific sgAC activity (Fig. 2). Greater threat-related activity was observed in men than in women in the right superior frontal gyrus and left postcentral gyrus. No sex differences were observed in amygdalar activation during threat. Voxel-based morphometry revealed no significant structural differences between men and women in any of the regions implicated in the functional analysis.

DISCUSSION

Greater fear-related activity in women of the sgAC, insula and brainstem indicates sex-divergent function of a brain network involved in autonomic and affective response to negative physical and emotional experience. The sgAC, part of the vmPFC, has extensive reciprocal connections to the amygdala, insula, brainstem and throughout the limbic system, and functions to integrate and respond to motivationally relevant internal and external information [17]. Previous functional neuroimaging studies in normal participants have shown that sgAC activity increases under conditions of negative emotion [17] and during extinction of a conditioned fear response [18]. Our finding of decreased

sgAC activity in men during anticipatory anxiety – the opposite pattern to that shown by women – is in accord with an earlier study using a similar paradigm in a predominantly male group [19]. SgAC activity has been considered to reflect a balance between the sometimes competing demands of emotion and cognition [17,19]; present results suggest that this balance may differ between men and women.

Previous reports have described increased sgAC activity in women as compared with men during pain [20] and in response to aversive pictures [10]. Notably, a recent metaanalysis of 65 functional neuroimaging studies identified, specifically, the sgAC and medial brainstem as regions more commonly activated in women than in men during emotional activation paradigms [9]. However, these structures have received scant attention in individual reports, perhaps because study hypotheses and analyses have to date been more focused on the amygdala.

Structural and functional sgAC abnormalities have been demonstrated consistently in patients with severe psychiatric disease, including depression and posttraumatic stress disorder [1–5], emphasizing the critical role this region plays in emotional regulation. On the basis of evidence that alterations in pregenual and subgenual anterior cingulate activity accompany, and in some cases predict, successful treatment for depression using a variety of therapeutic modalities (including medication, psychotherapy, surgery and placebo), chronic stimulating electrodes were recently implanted in the sgAC region of six patients with refractory depression, and found to induce sustained remission of depression in four [5].

The insula, activated in previous human fear-related studies [16,21], is involved in integrating external sensory stimuli such as pain with representations of internal bodily states, and is activated in correlation with autonomic measures of arousal. Brainstem activation in a region encompassing the superior colliculi, reticular activating system (RAS), dorsal raphe nucleus and midbrain periaqueductal gray (PAG) must be interpreted with caution given the paucity of functional imaging studies focused on the brainstem, and signal characteristics in this region, but may reflect low-level differential collicular visual processing of threat-related stimuli, PAG activity associated with pain expectation/analgesia or the fight/flight/freeze fear response, RAS-mediated increased arousal and/or increased serotonergic tone.

No significant sex differences in amygdalar activity were observed during threat. The amygdala is involved in fear conditioning [15,21] and instructed fear [16] and has been implicated in earlier functional imaging studies of sex differences under negative emotional conditions [7,8,11]. The absence of sex differences in amygdalar activity during threat in this study may relate to the evolutionarily essential role of this phylogenetically conserved structure, which might be expected to function similarly in men and women under dangerous conditions in order to maximize chances of survival. Previously reported sex differences in amygdala functioning, including the replicated finding of sex-dependent asymmetries in amygdalar activity associated with enhanced emotional memory for aversive stimuli [8,11], may relate to high-order, distinctly human neural processes such as conscious reflection upon or explicit memory for unpleasant experiences, perhaps reflecting sex differences in

evolutionarily newer neural connections between the amygdala and neocortical brain structures. On the basis of the current findings, however, the amygdala itself appears to function similarly in healthy men and women during instructed fear. Additional studies with larger numbers of participants would be needed to confirm this negative finding and ensure it is not due to insufficient statistical power.

In sum, this first report of human functional neuroanatomic sex differences during instructed fear/anticipatory anxiety extends and refines previous work through the use of a translational probe based on well-studied animal models, with psychological relevance to understanding human fear in the real world, where anxiety about what will or might happen arguably constitutes the major aspect of modern-day stress. Identification of sex-divergent sgAC functioning suggests a plausible neural substrate for an interaction between sex and psychological stress mediating susceptibility to psychiatric disease. In animal models, chronic stress has been shown to reduce dendritic size and number in vmPFC [22]. By analogy, we hypothesize that psychological stress – modeled here as anticipatory anxiety, which we have shown to be associated with elevated sgAC activity in healthy women – when severe, repetitive or occurring at a sensitive age, could induce or exacerbate sgAC pathology, perhaps via excitotoxic mechanisms, resulting in the persistent sgAC dysfunction demonstrated in multiple earlier studies of patients suffering from anxiety and affective disorders [1–5].

These results provide a neuroanatomical focus for further investigation of the role of sex in gene/environment interactions underlying the development of psychiatric disease, including findings that polymorphisms in a serotonin transporter gene are associated with both hyper-reactivity to negative stimuli in functional neuroimaging studies [23] and predisposition to depression following stressful life events [24]. Recent work in nonhuman primates suggests that certain manifestations of this polymorphism may differ between males and females [25]. Future human functional neuroimaging studies can identify neural intermediate phenotypes relevant to understanding interactions between sex, hormonal measures, genotype and behavioral/clinical phenotype.

CONCLUSION

Women demonstrated greater sgAC, insula and brainstem activity than men during instructed fear/anticipatory anxiety. On the basis of the role of sgAC and its disruption in psychiatric disorders and their treatment, this finding suggests a contributory neural substrate for the greater susceptibility of women to anxiety and affective disorders. These results emphasize the importance of considering sex when designing and interpreting functional neuroimaging studies.

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