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Gender Differences in Neural Correlates of Stress-Induced Anxiety

Dongju Seo^{a,e}, Aneesha Ahluwalia^b, Marc N. Potenza^{a,c,d,e}, and Rajita Sinha^{a,c}

^aPsychiatry, Yale School of Medicine, New Haven, CT

^bPsychology, Yale University, New Haven, CT

^cNeuroscience and Child Study Center, Yale School of Medicine, New Haven, CT

^dNational Center on Addiction and Substance Abuse (CASAColumbia), Yale School of Medicine, New Haven, CT

^eConnecticut Mental Health Center, New Haven, CT

Abstract

Although gender differences have been identified as a crucial factor for understanding stress-related anxiety and associated clinical disorders, the neural mechanisms underlying these differences remain unclear. To explore gender differences in the neural correlates of stress-induced anxiety, the current study utilized functional magnetic resonance imaging to examine brain responses in 96 healthy men and women with commensurable levels of trait anxiety as they engaged in a personalized guided imagery paradigm to provoke stress and neutral-relaxing experiences. During the task, a significant Gender main effect emerged, with men displaying greater responses in the caudate, cingulate gyrus, mid-brain, thalamus, and cerebellum. In contrast, women showed greater responses in the posterior insula, temporal gyrus, and occipital lobe. Additionally, a significant Anxiety Ratings $\times$ Gender interaction from whole-brain regression analyses was observed in the dorsomedial prefrontal cortex, left inferior parietal lobe, left temporal gyrus, occipital gyrus, and cerebellum ($p < 0.05$, whole-brain FWE corrected), with positive

Corresponding author: Rajita Sinha, Ph.D., Department of Psychiatry, Yale University School of Medicine, 2 Church Street South Suite 209, New Haven, CT 06519, Rajita.sinha@yale.edu, Telephone: 203-737-5805 Fax: 203-737-1272.

Conflicts of Interest

The authors do not have any financial or other interest or relationship that might be perceived to influence the results or interpretation of the manuscript. Dr. Potenza has: consulted for and advised Lundbeck, Ironwood, Shire, INSYS, RiverMend Health, Opiant/Lakelight Therapeutics and Jazz Pharmaceuticals; received research support from the National Institutes of Health, Veteran's Administration, Mohegan Sun Casino, the National Center for Responsible Gaming, and Pfizer pharmaceuticals; participated in surveys, mailings, or telephone consultations related to drug addiction, impulse control disorders or other health topics; consulted for law offices and the federal public defender's office in issues related to impulse control disorders; provides clinical care in the Connecticut Department of Mental Health and Addiction Services Problem Gambling Services Program; performed grant reviews for the National Institutes of Health and other agencies; has guest-edited journal sections; given academic lectures in grand rounds, CME events and other clinical/scientific venues; and generated books or chapters for publishers of mental health texts. Dr. Sinha has advised Embera Neurotherapeutics and RiverMend Health.

Author's role

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: RS, MP, DS. Acquisition of data: MP, RS. Analysis and interpretation of data: DS, AA, RS. Drafting of the manuscript: AA, DS. Critical revision of the manuscript for important intellectual content: DS, AA, MP, RS. Statistical analysis: DS, AA. Obtained funding: MP, RS, DS. Administrative, technical, and material support: MP, RS. Study supervision: MP, RS, DS.

associations between activity in these regions and stress-induced anxiety in women, but negative associations in men, indicating that men and women differentially utilize neural resources when experiencing stress-induced anxiety. The findings suggest that in response to stress, there is a greater utilization of the medial prefrontal-parietal cortices in experiencing subjective anxiety in women, while decreased utilization of this circuit was associated with increased subjective anxiety states in men. The current study has implications for understanding gender-specific differences in stress-induced anxiety and vulnerability to stress-related clinical disorders, and for developing more effective treatment strategies tailored to each gender.

Graphical abstract

In response to stress-induced anxiety, men and women displayed opposite patterns of neural activity in the dorsomedial prefrontal cortex, left inferior parietal lobe, left temporal gyrus, occipital gyrus, and cerebellum. The findings suggest that males and females differentially utilize neural resources when experiencing stress-induced anxiety; implications are discussed.

Keywords

stress; anxiety; gender difference; medial prefrontal cortex; parietal lobe

Introduction

Stress and related anxiety responses significantly contribute to the development of mental and physical disorders (Cohen *et al*, 2007; Larzelere and Jones, 2008). In particular, gender differences have been identified as a critical factor for understanding stress-related anxiety responses. Prior studies indicate that women tend to report higher levels of anxiety (Feingold, 1994) and a greater likelihood of expressing their emotions verbally (Brody and Hall, 1993), which may contribute to their increased vulnerability to emotional distress and related disorders (Bangasser and Valentino, 2014; Kessler *et al*, 1981).

However, it is unknown how men experience the same intensity of stress and anxiety; while men are less likely to verbally report distress and anxiety, they may tend to experience the anxiety at a more physiological level and engage in more action-oriented stress coping (Lindquist *et al*, 1997; Tamres *et al*, 2002). Some studies indicate that men and women may have an equivalent stress and anxiety-related vulnerability (Chaplin *et al*, 2008; Kessler *et al*, 1993), but this vulnerability may manifest in different psychiatric disorders. As such, women have a greater lifetime prevalence of stress-related psychiatric disorders such as depression

and anxiety disorders (Bangasser *et al*, 2014), while men have a greater prevalence of externalizing disorders (e.g., aggression, substance abuse) (Hicks *et al*, 2004). This suggests that men and women may utilize different neural resources in response to stress-related anxiety. However, thus far, the neural mechanisms underlying gender-specific anxiety responses remain unclear.

Prior data indicate that the medial prefrontal cortex (PFC) and amygdala contribute importantly to anxiety (Adhikari *et al*, 2010; Kim *et al*, 2011). The amygdala is activated during emotional distress, fear, and anxiety (Shin and Liberzon, 2010). The medial PFC has been found to be a critical regulator of emotion, stress and amygdalar responses (Diorio *et al*, 1993; Etkin *et al*, 2011; Ochsner *et al*, 2002; Urry *et al*, 2006), and amygdalar dysfunction has been associated with increased emotional distress and anxiety disorders (Myers-Schulz and Koenigs, 2012). However, most of this work has studied men and women together, and not systematically examined gender differences. (Seo *et al*, 2011b). Thus far, a handful of fMRI studies have examined sex-related differences. For example, studies have reported greater ventral PFC activation in response to stress in men (Seo *et al*, 2011b; Wang *et al*, 2007), and greater superior/middle frontal gyrus activity in women, which were also associated with in-scan stress anxiety (Seo *et al*, 2011b). While these findings are informative, these studies did not match trait anxiety levels in men and women prior to the neuroimaging session. One anxiety-matched study found sex differences with greater response in men in the medial PFC and limbic regions, but did not report specific neural correlates of stress-related anxiety ratings (Goldstein *et al*, 2010). Thus far, sex differences in neural responses to stress-induced anxiety have not been reported, especially in individuals with similar trait anxiety levels. Therefore, it remains unclear whether men and women utilize similar circuitry that is differentially activated, based on intensity of stress, or if they use different circuitry, which would imply gender-specific vulnerability to stress anxiety and coping.

The present study aims to investigate sex differences in neural responses to acute stress exposure and the neural correlates of stress-induced anxiety in healthy men and women with similar trait anxiety levels. To manipulate a state of acute stress, we utilized a well-validated, commonly used method of emotion and stress provocation that involves a personalized guided imagery task (Sinha, 2009). On-line measures of anxiety ratings were concurrently collected during stress exposure. Based on prior findings (Goldstein *et al*, 2010; Seo *et al*, 2011b; Wang *et al*, 2007), we hypothesized that males and females would differ in medial PFC and limbic activity in response to stress and stress-induced anxiety.

Materials and Methods

Participants

Participants included 96 healthy, right-handed individuals (41 women and 55 men) between the ages of 19 and 50 years who were recruited from the community via flyers and newspapers. They completed demographic, cognitive, psychiatric, and drug-use assessments over the course of 2-3 sessions. All participants completed the State-Trait Anxiety Inventory (STAI) (Spielberger, 1985) and perceived stress scale (PSS) (Cohen *et al*, 1983), common measures of anxiety and perceived stress respectively. Participants were excluded if they

had, a history of head trauma, use of psychoactive medication, current or lifetime substance abuse or dependence, current or lifetime neurological or psychiatric disorders (as determined by the Structured Clinical Interview for DSM-IV), or were pregnant at the time. Women were scheduled to participate in the fMRI sessions during the luteal or follicular phases of their menstrual cycle to control for the possible influence of steroid hormonal fluctuations on stress responses (Kirschbaum *et al*, 1999). Women were excluded if they were using hormonal birth control or reported irregular menstrual cycles. The study procedures were approved by the Human Investigation Committee at the Yale University School of Medicine, and informed consent was signed by all participants prior to participation.

Guided Imagery Script Development

In a session prior to the fMRI scan, personalized imagery scripts were developed from participants' descriptions of two neutral-relaxing experiences and two stressful experiences, using the Scene Construction Questionnaires (Abel *et al*, 2010; Britton *et al*, 2005; Li *et al*, 2005b; Sinha *et al*, 2009). This script-based individualized imagery method is an established and well-validated method (Sinha, 2009; Sinha and Tuit, 2012) that has been used in numerous studies for stress and emotion induction (Cooney *et al*, 1997; Fox *et al*, 2012; Fox *et al*, 2007; Hommer *et al*, 2012; Jastreboff *et al*, 2011; Li *et al*, 2005a; Potenza *et al*, 2012; Seo *et al*, 2011a; Sinha *et al*, 2009; Sinha *et al*, 2011). Scripts were initially developed and used for three conditions (stress, neutral-relaxing, reward) in an fMRI task. However, only the stress and neutral-relaxing conditions were examined, as only these conditions were relevant to the aims of the current study (i.e., to examine gender-specific neural correlates of stress-induced anxiety). As expected, anxiety ratings did not increase during reward trials, showing no significant difference from the neutral-relaxing condition, supporting the rationale that there is minimal reward-related anxiety in health individuals and thus, the reward condition is not relevant to understanding neural correlates of anxiety. To develop the stress scripts, participants were asked to recount situations that they experienced in the past year that were particularly stressful and over which they had no control in the moment of their occurrence. This includes highly stressful situations such as unemployment, loss of a loved one, relationships problems, etc. Participants rated the situations on a 10-point Likert scale (1 = not at all stressful and 10 = the most stressful), and only stressful situations that were rated an 8 or above were used for script development, thereby providing an individualized calibration of the stressful experience. Neutral-relaxing scripts were based on personal accounts of commonly experienced neutral-relaxing situations, such as taking a walk or laying on the beach. Although personal content specific to a participant's experience was included in each script, the script format, style and length were standard across conditions and subjects, as outlined by previously described guidelines (Sinha *et al*, 2009). Scripts were each 2.5-minutes in length, and were audio-taped to be presented in random, counterbalance order during the scanning session.

Efficacy of Script-driven Imagery Manipulation

The following procedures were implemented to ensure the effectiveness of the imagery manipulation. First, all participants completed the Questionnaire on Mental Imagery (QMI), a measure of individual differences in mental imagery ability (Sheehan, 1967). The QMI scores of female participants ($M=72.0$, $SD=26.5$) did not differ significantly from those of

male participants ($M=74.4$, $SD=23.8$). Prior to the scanning session, individuals participated in a structured relaxation and imagery training procedure that is known to minimize variability in imagery ability (Sinha, 2009). Then, following each of the stress and neutral-relaxing trials, each participant rated imagery vividness on a 10-point Likert scale (1=cannot visualize the image and 10=extremely clear, 'as if' it were happening right now). A general linear model with condition (stress/neutral-relaxing) as the within-subjects factor and gender as the between-subjects factor showed no significant gender main effect or a gender-by-condition interaction in vividness ratings. However, there was a significant condition main effect ($F(1, 94) = 8.1$, $n = 96$, $p < 0.01$) with greater ratings for the stress condition ($M=8.3$, $SD=1.2$) compared to the neutral-relaxing conditions ($M=8.0$, $SD=1.3$) ($t=2.8$, $p < 0.01$).

fMRI Acquisition

Images were collected with the 3-T Siemens Trio MRI system, which is equipped with a single-channel, standard-quadrature head coil. A T2*-sensitive gradient-recalled single shot echo-planar pulse sequence was used. Anatomical images were obtained with spin-echo imaging in the axial-plane parallel to the AC-PC line (repetition time (TR) = 300 msec, echo time (TE) = 2.5 msec, bandwidth = 300 Hz/pixel, flip angle = 60 degrees, field-of-view = 220×220 mm, matrix = 256×256 , 32 slices, slice thickness = 4mm, no gap). A single-shot gradient echo-planar imaging sequence was utilized to acquire functional images. It consisted of 32 axial slices parallel to the AC-PC line covering the whole brain (TR = 2,000 msec, TE = 25 msec, bandwidth = 2004 Hz/pixel, flip angle = 85 degrees, field of view = 220×220 mm, matrix = 64×64 , slice thickness = 4mm with no gap). After functional image collection, sagittal images for multisubject registration were obtained by via high-resolution 3D Magnetization Prepared Rapid Gradient-Echo sequence (TR = 2530 ms; TE = 3.34 ms; bandwidth = 180 Hz/pixel; flip angle = 7° ; slice thickness = 1mm; field-of-view = 256×256 mm; matrix = 256×256).

fMRI Task and Collection of Online Anxiety Ratings

A block design was used with each fMRI block lasted a 5-minute (min). Each 5 min block consisted of a 1.5-min baseline period, a 2.5-min imagery period (2-min of read-imagery and 0.5-min of quiet-imagery) and a 1-min quiet recovery period. During the baseline period, participants were asked to stay still in the scanner without any mental activity. During the recovery, they remained still while recovering from the imagery period. The order of script presentation was counterbalanced and randomized across subjects. No script was presented more than once, and scripts from the same condition were never presented consecutively. Participants used a 10-point verbal analog scale (1=not at all, 10=extremely high) to rate their anxiety levels before and after each fMRI trial. Ratings referred to how "anxious, tense and/or jittery" they felt. Between fMRI trials, participants engaged in a 2-min progressive relaxation to normalize residual emotion from previous trials, which did not involve mental imagery or fMRI image acquisition. Heart rate data were concurrently collected using a pulse oximeter.

fMRI Analysis

XMedCon was used to convert the raw imaging data from Digital Imaging and Communication in Medicine format to Analyze format (Nolfe, 2003). The first ten images

were discarded from the beginning of each functional run in order to attain a steady-state equilibrium between radio-frequency pulsing and relaxation. The recovery period was not included in the data analysis to exclude carryover effects from the imagery phase. Images were motion-corrected for three translational and three rotational directions (Friston *et al*, 1996). Trials with a linear motion exceeding 1.5 mm or a rotation greater than 2 degrees were discarded; two out of 192 stress trials (from female) and two out of 192 neutral-relaxing trials (1 from female, 1 from male) were removed. At the individual level, General Linear Model (GLM) was used with a regressor (time during imagery compared to the baseline) on each voxel in the entire brain volume for each trial (stress-baseline and neutral-relaxing-baseline). A 6 mm Gaussian kernel was used to spatially smooth each functional trial, and they were individually normalized to create beta-maps ($3.44\text{mm} \times 3.44\text{mm} \times 4\text{mm}$). The Yale BioImage Suite software package (Duncan *et al*, 2004; Papademetris *et al*, 2006) was used to sequentially conduct three registrations to adjust for individual anatomical differences: linear registration of raw data into 2D anatomical image, the 2D to 3D ($1 \times 1 \times 1$ mm) linear registration, and a non-linear registration to a reference 3D image. The reference image used was the Colin27 Brain (Holmes *et al*, 1998) in Montreal Neurological Institute (MNI) space (Evans *et al*, 1993).

For the second level group analysis, random mixed-effect models were implemented using the Analysis of Functional NeuroImages (AFNI) software (Cox, 1996). A gender-by-condition 2×2 ANOVA was performed, using condition (stress/neutral-relaxing) as the within-subject fixed-effect factor, gender (women/men) as the between-subject fixed-effect factor, and subject as the random-effect factor using the AFNI 3dLME program. A FamilyWise Error rate (FWE) correction was applied for multiple comparisons with Monte Carlo simulations (Xiong *et al*, 1995) using AlphaSim in AFNI (Cox 1996). The AlphaSim was implemented with a smoothing kernel of 6 mm and a connection radius of 6.296mm on $3.44\text{mm} \times 3.44\text{mm} \times 4\text{mm}$ voxels, and determine that an activation volume of 231 original voxels (6248 mm^3) met the $p < 0.05$ corrected threshold. In addition, to understand the relationship between brain activity during stress-neutral-relaxing and stress-induced anxiety ratings, whole-brain regression analysis was performed using AFNI with gender included as a covariate with the application of FWE correction for multiple comparisons.

Results

Demographics

Table 1 presents the demographic characteristics of all 96 participants and of participants by gender. There were no significant differences between men and women in terms of age, race, education, BMI, or state or trait anxiety as measured by the STAI (Spielberger, 1985) and levels of perceived stress as measured by perceived stress scale (Cohen *et al*, 1983).

Anxiety Ratings and Heart Rate

Gender effects on the on-line self-report measure of anxiety were assessed using the general linear model with condition (stress/neutral-relaxing) as the within-subjects factor and gender as the between-subjects factor. There was no significant Gender main effect or Gender $\times$ Condition interaction in anxiety ratings. However, there was a significant condition main

effect ($F(1, 94) = 130.6, n=96, p<0.001$). As expected, for all subjects, the anxiety ratings in the stress imagery condition ($M = 3.95, SD = 2.3$) were significantly higher than those in the neutral-relaxing imagery condition ($M = 1.61, SD = 1.63$) ($t = 11.72, p < .0001$) (see Figure 1a), indicating successful induction of a stress state. For heart rate, a general linear model was implemented with condition (stress/neutral-relaxing) as the within-subjects factor and gender as the between-subjects factor. The results indicated a significant main effect of condition ($F(1, 89) = 41.9, n = 91, p<0.0001$) and gender ($F(1, 89) = 8.6, n = 91, p<0.01$), but no gender-by-condition interaction. Heart rate response was greater for women ($M=72.3, SD=12.5$) than men ($M=65.6, SD=9.5$) ($t=2.9, p<0.01$). In addition, the response was greater for the stress condition ($M=70.1, SD=12.5$) than the neutral-relaxing condition ($M=66.7, SD= 10.5$) ($t=6.6, p<0.0001$) (see Figure 1b). Neither gender group displayed associations between anxiety ratings and heart rate responses during the task in either condition.

Task-related Brain Activity

A 2×2 LME identified significant main effects of Task and Gender, but no Gender $\times$ Task interaction ($p<0.05$, whole-brain FWE corrected). A significant Task main effect on brain activation was observed in regions including the medial PFC, precuneus, anterior and posterior cingulate cortex (ACC/PCC), lateral prefrontal cortex (LPFC), temporal gyrus, thalamus, insula, right amygdala and striatum (see Figure 2); all of these areas displayed greater activation in the stress condition than in the neutral-relaxing condition in all individuals. No area was more active in the neutral-relaxing condition relative to the stress condition. A significant Gender main effect was evident (see Figure 3). Compared to women, men displayed greater levels of activity in the striatum, ACC/PCC, mid-brain regions, thalamus, mid-brain and parts of the cerebellum. However, women, compared to men, showed greater activation in the posterior insula, superior/middle temporal gyrus, and occipital lobe (Figure 3).

Whole-brain Regression Analysis

Whole-brain regression analysis showed significant Gender $\times$ Anxiety interaction with no Anxiety main effect when all men and women are considered, with significant Gender $\times$ Anxiety interactions coming from the dorsomedial PFC, precuneus, left inferior parietal lobe (IPL), left superior/middle temporal gyrus, occipital gyrus, and cerebellum during the stress-versus-neutral-relaxing contrast (see Figure 4, Table 2; $p < .05$, whole-brain FWE corrected). In order to understand the source of interaction effects, post-hoc regression analyses for simple effects were conducted separately for men and women (see Figure 5 and Table 2). Stress-induced anxiety predicted increased activity in women (standardized coefficient (β) = 0.38) and decreased activity in men ($\beta = -0.33$) in the dorsomedial PFC. Similarly, precuneus/IPL activity was associated with stress-induced anxiety positively in women ($\beta = 0.33$) and negatively in men ($\beta = -0.40$). Likewise, activity in the temporal gyrus was positively associated in women ($\beta = 0.44$) and negatively in men ($\beta = -0.3$). Activity in a cluster involving the occipital lobe and cerebellum was also associated with stress-induced anxiety positively in women ($\beta = 0.40$) and negatively in men ($\beta = -0.36$) (all p 's $< .05$, whole-brain FWE-corrected). There were no outliers in any of these regions. (see Figure 5, Table 2; $p < .05$, whole-brain FWE corrected).

Discussion

The current study examined gender differences in neural correlates of online stress-induced anxiety response in men and women with commensurable levels of STAI anxiety and perceived stress. We report gender-specific neural correlates of anxiety during stress provocation, mainly in the medial prefrontal and parietal cortices, with opposite patterns of associations in men and women. The observed neural difference indicates that men and women differentially utilize neural resources when experiencing anxiety during stress.

Gender Main Effect

In task-related activity, a significant Gender main effect was observed in cortico-striatal regions: men displayed a greater response in the caudate, ACC/PCC, mid-brain, thalamus, and parts of the cerebellum, while women showed a greater response in the posterior insula, right temporal gyrus, and occipital lobe. However, no Gender $\times$ Task interaction was observed, suggesting that there are sex differences in neural responses to a general imagery experience, but not specifically to the stress or neutral-relaxing condition. Specifically, males displayed greater activation in the caudate, midbrain, ACC, and thalamus—brain areas involved in instrumental action and in motor functions. The caudate plays a role in motor processes, planning and execution of movement, and goal-directed action (Grahn *et al*, 2008), and the midbrain functions in motor movement as well (Boecker *et al*, 2008). The ACC is involved in action selection, error correction, and behavioral adjustment (Shenhav *et al*, 2013). Additionally, the thalamus functions as a motor relay center (Sommer, 2003), and the cerebellum also contributes importantly to motor control (Paulin, 1993), with some involvement in cognitive and emotional processing (Schmahmann, 2004; Stoodley and Schmahmann, 2009).

On the other hand, females exhibited increased activity in the occipital lobe, right temporal gyrus, and insula—regions that are associated with visual processing, verbal expression, and emotional experience respectively (Golby *et al*, 2001; Grill-Spector *et al*, 1998; Singer *et al*, 2009). The increased activity in the visual cortex and temporal gyrus indicates that, compared to men, women may be more likely to engage in visualizing their experiences and semantic processing during imagery experiences. The insula is related to emotional arousal and interoceptive processing (Singer *et al*, 2009), suggesting greater emotional and interoceptive activity during imagery in women. In addition, women displayed greater heart rate responses than men, regardless of condition, replicating results of prior studies (Agelink *et al*, 2001; Ryan *et al*, 1994). Higher heart rate has been linked to greater emotional response via autonomic nervous system arousal (Everhart and Harrison, 2002; Sinha *et al*, 2009). Elevated heart rate is also often associated with emotional disorders including depression (Lahmeyer and Bellur, 1987). Taken together, these findings suggest that during guided imagery women may experience emotions and feelings via more sensory and semantic processing in the brain and greater autonomic nervous system arousal.

Gender differences in neural correlates of stress-induced anxiety

Gender differences were evident in the neural correlates of stress-induced anxiety, even when experiencing similar levels of anxiety during stress. During stress exposure, men and

women displayed opposite patterns of associations in the dorsomedial PFC, parietal cortices (including left IPL), left temporal gyrus, occipital cortices and cerebellum regions, where stress-induced anxiety was associated with greater neural activity in women, but with decreased neural activity in men, in each of these regions. These findings suggest that women experiencing anxiety during acute stress may have difficulty regulating hyperactivity in these regions. On the other hand, men may experience increased anxiety in response to stress because of hypoactivation, or even suppression, of these brain regions.

Differential association with stress-induced anxiety by gender was observed in the dmPFC and IPL—regions that are involved in high-level cognitive operations. Activity in the dmPFC is related to higher order executive functions and cognitive control (Seo *et al*, 2014), as well as cognitive awareness of anxious emotions (Etkin, 2010). Additionally, this region has been found to be associated with strategy-oriented reasoning (Seo *et al*, 2014), and is involved in high-level cognitive operations and regulation of response strategies (Matsuzaka *et al*, 2012). Similarly, increased activity in the left IPL has been associated with greater cognitive reappraisal and response strategies (Seo *et al*, 2014). Meanwhile, the precuneus is involved in self-conscious and self-referential thinking (Cavanna and Trimble, 2006). These studies along with our findings suggest that differential activation and engagement of these cognitive processing regions is associated with gender-specific anxiety experience during stress. Men and women may differ in the extent to which they engage in cognitive, self-conscious and referential processes, which in turn may contribute to their differential subjective anxiety experiences.

Consistent with the above interpretation, evidence suggests that the medial PFC, including the dmPFC, is involved in self-referential thinking, rumination and depression (Cooney *et al*, 2010; Nejad *et al*, 2013). Thus, the higher levels of dmPFC activation associated with stress-induced anxiety in females suggest that they may have a greater susceptibility to excessive stress-consistent cognitive processing, including possibly negative cognitions related to the acute stress experience. Consistent with this notion, previous evidence indicates that females have a greater tendency to ruminate than males (Johnson and Whisman, 2013), which could increase their risk for depression and anxiety disorders relative to that of males (Nolen-Hoeksema *et al*, 1999). Together, the observed results indicate that, compared to men, higher stress-induced anxiety in women may be related to over-engagement of cognitive processing during stressful events.

In contrast, stress-induced anxiety in males was associated with decreased reactivity in the dmPFC, precuneus, and IPL. A preclinical study found that hypo-reactivity to stress in the dmPFC is associated with a greater anxiety response in male rats (Kalisch *et al*, 2004). This study proposed that the failure to sufficiently activate the dmPFC in response to stress could contribute to high levels of anxiety. In a similar vein, a study on individuals with anxiety disorders concluded that adequate activation of the dmPFC is beneficial for reducing anxiety (Goldin *et al*, 2013). Additionally, EEG studies indicate that a reduced P300 response, which was collected from parietal scalp locations, was associated with externalizing behaviors in men, including aggression and substance abuse (Patrick *et al*, 2006). Furthermore, an fMRI study found a negative association between IPL activity and violent behavior in men (Kumari *et al*, 2006), suggesting vulnerability associated with reduced parietal function in

men. Overall, the decreased parietal lobe and dmPFC activity observed in the current study could indicate that males have a hypoactive response to stress in regions involved in utilizing cognitive resources or strategies when experiencing stress-induced anxiety, which may in turn facilitate the tendencies to engage in more externalizing behaviors under stress.

The present study also found gender-specific associations with stress-induced anxiety in sensory-motor regions including the left temporal gyrus, occipital cortices and cerebellum. Prior studies indicate that the left temporal gyrus is involved in processing semantic information and verbal stimuli (Friederici *et al*, 2003; Golby *et al*, 2001). Along with increased activity in the visual cortex associated with stress-induced anxiety, the observed patterns may suggest that females engage in more verbal and visual mental activity while experiencing stress, which could enhance their anxiety. The cerebellum is also known to be involved in cognitive and emotional processing (Schmahmann, 2004; Stoodley *et al*, 2009). During emotional processes, cerebellar activity tends to be synchronized with cortical activity (Krienen and Buckner, 2009), suggesting that differential cerebellar activity likely reflects cortical activity (e.g., dmPFC, parietal lobe) in response to stress-induced anxiety. Taken together, the observed differences in these regions suggest that men and women may differ in the extent to which they engage in verbal processing, visualization, self-referential thinking, and cognitive processing during the experience of stress and anxiety.

Conclusions and implications

In conclusion, the current study reports that men and women differentially utilize neural resources when experiencing stress-induced anxiety. The observed differences may help develop a better understanding of gender-specific stress coping and vulnerabilities to certain stress-related disorders. Supporting this, gender specific stress management training has been implemented with promising results—greater relaxation in women and less externalizing coping in men (Hampel *et al*, 2008; Matud, 2004). Specifically, anxious women may benefit from practicing mindfulness, a validated stress management tool that helps reduce stress and anxiety (Deyo *et al*, 2009; Zeidan *et al*, 2014). fMRI studies have demonstrated that practicing mindfulness meditation results in decreased activation of the medial PFC and parietal cortices (Barnby *et al*, 2015; Brewer *et al*, 2011), regions that the current study found to be hyperactive in women while experiencing stress-induced anxiety. Men may benefit from learning strategies, such as cognitive behavioral therapy (CBT) (Lindquist *et al*, 1997); there is evidence that CBT enhances activity in the IPL and dmPFC in response to anxiety-inducing situations (Goldin *et al*, 2013; Goldin *et al*, 2014), regions that were hypoactive in men during stress anxiety in our study.

Although the current findings have some implications for gender-specific management for anxiety and stress-related disorders, it is important to note that our sample comes from a healthy, community population without clinical levels of anxiety. Therefore, the results should be interpreted with caution when being applied to clinical samples. Nonetheless, our findings provide valuable information as the stress-related neural patterns found in this study may indicate a gender-specific prodromal pattern of stress-related anxiety disorders. Limitations also include the cross-sectional nature of the study, and, thus, further studies are needed to examine exact associations between gender-specific neural correlates and

vulnerability to psychiatric disorders later in life. It is of note that literature suggests that the stress response in females may vary by menstrual cycle phase, with greater responses observed in luteal vs. follicular phases (Collins *et al*, 1985; Kajantie and Phillips, 2006; Kirschbaum *et al*, 1999; Ossewaarde *et al*, 2010). To clarify the potential influence of the hormonal milieu of woman in gender differences, further studies are needed to examine neural differences in demographically matched women from follicular and luteal phases. In addition, future studies could benefit from investigating gender-specific neural response in individuals with clinical levels of stress and anxiety disorders.

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Significance Statement

Gender differences play a crucial role in understanding stress-related anxiety and associated clinical disorders. To investigate neural mechanisms underlying gender difference in stress-induced anxiety, the current study utilized functional magnetic resonance imaging in 96 healthy men and women with commensurable levels of anxiety during stress-inducing and neutral-relaxing guided imagery. Opposite patterns (positive in women, but negative in men) of associations with stress-induced anxiety were observed for activity in the dorsomedial prefrontal cortex, left parietal and temporal lobes, occipital gyrus, and cerebellum. Men and women may differentially utilize neural resources when experiencing stress-induced anxiety, and this could underlie the well-known gender-specific vulnerabilities to stress-related clinical disorders.

Figure 1.

Anxiety ratings (a) and heart rate responses (b) in stress and neutral-relaxing imagery conditions by gender. Means and standard errors (SE) are shown for men and women for verbal analog scales (0-10) assessing subjective anxiety for the stress and neutral-relaxing imagery conditions (a). Heart rate data are shown for the stress and neutral-relaxing imagery conditions (b). For both men and women, anxiety ($p < 0.001$) and heart rate response ($p < 0.0001$) in the stress imagery condition was significantly greater than anxiety and heart rate response in the neutral-relaxing imagery condition. There were no significant differences in anxiety ratings by gender and in heart rate response by gender. A main effect of gender was only found in heart rate response and not in anxiety ratings. Heart rate responses were significantly greater for women than men across conditions ($p < 0.01$). ** = $p < 0.001$; * = $p < 0.01$

Figure 2.

Brain regions showing significant Task main effect (Stress, Neutral-relaxing) from whole-brain voxel-based, fMRI linear mixed effects (LME) analyses. Stress-induced increases in fMRI signal relative to brain responses in the neutral-relaxing condition are shown in regions including the medial prefrontal cortex (mPFC), precuneus, posterior cingulate cortex (PCC), ventrolateral prefrontal cortex (VLPFC), middle temporal gyrus (MTG), middle frontal gyrus (MFG), thalamus, insula, right amygdala and caudate. There are no brain regions showing greater activity during the neutral-relaxing condition than the stress condition. L=left; R=right; 0.05 whole-brain corrected.

Figure 3.

Brain regions showing significant Gender main effect from whole-brain voxel-based fMRI linear mixed effects (LME) analyses. Women displayed greater activity in regions of posterior insula (P. insula), occipital lobe, and superior temporal gyrus (STG), while men showed greater activity in caudate, thalamus, anterior/posterior cingulate cortex (ACC/PCC), and cerebellum. Yellow and red colors indicate brain areas showing increased activation in women compared to men, while blue and purple colors indicate increased activation in men than women. 0.05 whole-brain FWE corrected.

Figure 4. Brain regions showing significant Gender-by-Anxiety interaction effect in the whole-brain regression analysis. Associations between stress-induced anxiety ratings and Stress-Neutral-relaxing brain activation show gender-specific effects in the dorsomedial PFC (dmPFC), left inferior parietal lobe (IPL), occipital lobe, middle temporal gyrus (MTG), and cerebellum (whole-brain FWE corrected at 0.05). Figure 5 shows the separate beta values and corresponding scatterplots for ROIs of each cluster in men and women, which are also indicated in Table 2. $p < .05$, whole-brain FWE corrected.

Figure 5.

Scatter plots and regression lines for stress-induced anxiety ratings with neural responses (Stress (S) - Neutral-relaxing (N)) for specific regions of interest (ROIs). Simple effects in ROIs from whole-brain regression of Gender-by-Anxiety interaction effects analyses from Figure 4 are shown separately in men and women. Stress-induced anxiety predicted brain responses to stress based on gender. The plots show (A) positive (women (W)) and negative (men (M)) associations between stress-induced anxiety ratings and activity in the dorsomedial PFC (W: $\beta = .38$, M: $\beta = -.33$), precuneus and inferior parietal lobe (IPL) (W: $\beta = .33$, M: $\beta = -.40$), middle/inferior temporal gyrus (W: $\beta = .44$, M: $\beta = -.30$), and occipital lobe and cerebellum (W: $\beta = .40$, M: $\beta = -.36$). Beta (β) indicates the standardized coefficient. There were no outliers in any of these brain regions for both men and women.

Table 1

Demographic characteristics of participants

Subject Variable	All (N = 96)	Women (N = 41)	Men (N = 55)	p value
Age	27.09 (7.82)	27.1 (6.28)	27.09 (8.85)	1
Race (N %)				
Caucasian	68 (71%)	23 (56%)	45 (81.8%)	0.08
Years of Education	15.08 (2.04)	14.95 (2.04)	15.18 (2.05)	0.59
BMI	26.35 (4.53)	26.26 (4.76)	26.42 (4.39)	0.86
STAI ^a				
Trait Anxiety	34.51 (10.3)	34.8 (11.13)	34.29 (9.73)	0.81
State Anxiety	34.26 (10.48)	35.98 (11.98)	32.97 (9.1)	0.17
Perceived Stress ^b	19.99 (8.71)	21.46 (9.83)	18.87 (7.66)	0.15

Note: No significant differences between men and women for any measure.

^aState-Trait Anxiety Inventory (Spielberger, 1985)

^bPerceived Stress Scale (Cohen et al., 1983)

Table 2

Brain regions showing significant interaction effect (Sex X Anxiety) from regression analysis ($p < 0.05$, whole-brain corrected)

Neural Correlates	Lat	Volume (mm ³)	Coordinates			F	Beta	
			X	Y	Z		Women	Men
DmPFC/MCG	B	7427	11	36	40	5.4	0.38	-0.33
Precuneus/IPL/PG	L	11776	-32	-44	57	5.7	0.33	-0.40
Middle/inferior Temporal G.	L	8078	-48	-58	-2	7.1	0.44	-0.30
Occipital lobe/Cerebellum	B	26259	-11	-70	-23	5.5	0.40	-0.36

Results from whole-brain regression analysis (during Stress-Neutral) with stress-induced anxiety Mean F and beta values and corresponding coordinates are denoted. Lat=laterality; L=Left; B=bilateral; DmPFC=dorsomedial prefrontal cortex; MCG=middle cingulate gyrus; IPL=inferior parietal lobe; PCG = precentral gyrus; G=gyrus. There was no outlier in any of brain clusters in Table 2. Beta indicates the standardized coefficient.