Sex-Related Differences in IBS Patients: Central Processing of Visceral Stimuli

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See editorial on page 1975.

Background & Aims: **Women have a higher prevalence of irritable bowel syndrome (IBS) and possible differences in response to treatment, suggesting sex-related differences in underlying pathophysiology. The aim of this study was to determine possible sex-related differences in brain responses to a visceral and a psychological stressor in IBS.** ***Methods:*** **Regional cerebral blood flow measurements using $H_2{}^{15}O$ positron emission tomography were compared across 23 female and 19 male nonconstipated patients with IBS during a visceral stimulus (moderate rectal inflation) and a psychological stimulus (anticipation of a visceral stimulus).** ***Results:*** **In response to the visceral stimulus, women showed greater activation in the ventromedial prefrontal cortex, right anterior cingulate cortex, and left amygdala, whereas men showed greater activation of the right dorsolateral prefrontal cortex, insula, and dorsal pons/periaqueductal gray. Similar differences were observed during the anticipation condition. Men also reported higher arousal and lower fatigue.** ***Conclusions:*** **Male and female patients with IBS differ in activation of brain networks concerned with cognitive, autonomic, and antinociceptive responses to delivered and anticipated aversive visceral stimuli.**

Women disproportionately experience a number of chronic disorders, including affective disorders, pain disorders, and functional visceral disorders such as irritable bowel syndrome (IBS).[1–3] Female-to-male prevalence ratios for IBS vary from 1:1 to >2:1 across a variety of studies,[4] and women are more likely to develop IBS-like symptoms following an episode of infectious gastroenteritis.[5] Although some of this sex-related bias has been attributed to differences in psychosocial factors and use of health care, considerable evidence is consistent with physiologic sex-related differences in the autonomic and perceptual response to pain and stress, which may contribute to the differences in prevalence.[6,7]

Although perception thresholds during rectosigmoid distention tend to be higher in healthy female controls compared with male controls, they are lower in female patients with IBS compared with male patients.[6] Despite similar disease severity, female patients with IBS report a greater frequency of nonpainful extraintestinal symptoms compared with male patients with IBS,[8] and IBS shares significant comorbidity with other female-predominant functional disorders such as fibromyalgia, migraine, and interstitial cystitis.[9] In addition, recent clinical trials with 2 new drugs for IBS aimed at different serotonin receptor subtypes have shown significant efficacy only in female patients with IBS.[10,11]

These observations suggest that there may be significant sex-related differences in the physiologic and behavioral response to aversive stimuli, specifically in the response to aversive stimuli arising from the pelvic viscera. It has been suggested that female-specific antinociceptive systems have evolved to minimize pain associated with events related to reproduction, such as menstrual cycle, intercourse, pregnancy, and delivery.[12] These systems would be targeted primarily at pelvic viscera, and a breakdown of these systems (such as decreased periaqueductal gray [PAG] activation) would be expected to result in greater pain sensitivity of pelvic viscera, including the rectosigmoid. In addition to these sex-specific responses to pain, an extensive literature supports sex-related differences in the physiologic and behavioral response to stressful stimuli in general.[7] It has been

Abbreviations used in this paper: ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; EMS, emotional motor system; IBS, irritable bowel syndrome; LC, locus ceruleus; PAG, periaqueductal gray; PET, positron emission tomography; PFC, prefrontal cortex; rCBF, regional cerebral blood flow; ROI, region of interest.

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suggested that, whereas men show a predominant fight-or-flight pattern (with predominant cardiovascular sympathetic activation, skeletomotor responses, and somatic antinociceptive responses), women may show an additional tend-and-befriend response to certain stressors that is aimed at counteracting the fight-or-flight pattern, favoring vagal responses and specific neuroendocrine responses involving oxytocin, opioids, and estrogen.[7]

To date, there is little evidence of sex-related differences in central responses to visceral stimuli from brain imaging studies. In a previous study, using rectal distention of aversive intensity, we reported greater activation of the insular cortex in male patients with IBS compared with female patients with IBS, suggesting possible differences in the processing of or autonomic response to aversive afferent information from the pelvic viscera.[13] In contrast, a recent functional magnetic resonance imaging study in healthy control subjects using rectal stimuli close to the perception threshold reported greater activation of insular and anterior cingulate cortices in female subjects.[14]

We have also recently shown in a combined male/female group of patients with IBS that the 5-HT_3 receptor antagonist alosetron decreases regional cerebral blood flow (rCBF) in the amygdala, ventromedial prefrontal cortex (PFC) and brain stem structures in the region of the PAG, locus ceruleus (LC) complex, and parabrachial nucleus.[15] These brain regions are part of the emotional motor system (EMS), a set of parallel output systems mediating the response to emotional stimuli, including fear and stress.[16,17] The EMS receives descending input from cortical areas (ventromedial PFC, anterior cingulate cortex [ACC]) during psychological stressors and ascending input from the viscera during aversive visceral stimulation.

In the current study, we aimed to test the general hypothesis of sex-related differences in the responsiveness of EMS circuits to both descending and ascending inputs related to aversive visceral experiences. These circuits are shown schematically in Figure 1. Specifically, we wanted to test the following in patients with IBS. First, are there sex-related differences in activation of brain regions that are part of the central autonomic, antinociceptive, and skeletomotor response circuits, consistent with male/female differences in behavioral responses?[6,7,18] Second, are these differences specific to rectal distention (gut-directed stimulus), or are they also seen in the mere anticipation of such a stimulus (central nervous system–directed stimulus)? We investigated these hypotheses using a standard visceral distention procedure[19] and $H_2{}^{15}O$ positron emission tomography (PET).

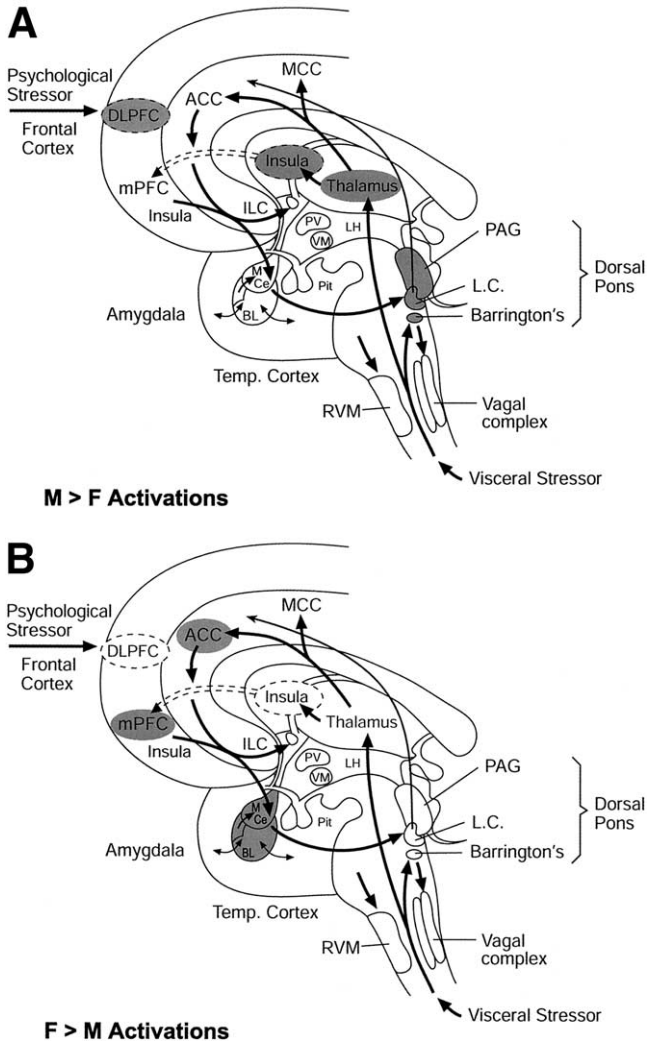

Figure 1. Schematic of brain circuits activated by visceral and central nervous system–directed stimuli. *Gray highlighted areas* indicate brain regions showing greater activation in (*A*) male patients and (*B*) female patients.

Patients and Methods

Patients

Data were analyzed from 42 right-handed patients with IBS (23 female and 19 male patients) recruited primarily by advertisement. Before screening, all patients gave informed consent according to the principles of the Declaration of Helsinki and in compliance with Food and Drug Administration requirements. To be included in the investigation, patients were required to meet Rome I criteria for IBS[20] and undergo clinical and endoscopic verification that they did not have inflammatory or other structural intestinal disease. All subjects were free from centrally neuroactive medications for at least 30 days preceding PET, and none had a history of psychiatric illness or substance abuse. All female volunteers had negative pregnancy test results.

Ten of the female patients were premenopausal, 3 were perimenopausal, and 10 were postmenopausal. The clinical

Table 1. Clinical Characteristics

		Mean	SD	M vs. F
Age	M	39.82	10.82	NS
	F	41.46	9.51	
Symptom severity (*6 mo*)	M	12.67	2.55	NS
	F	13.40	2.46	
Symptom severity (*24 h*)	M	7.17	4.66	NS
	F	7.78	4.75	
SCL90R General Severity Index	M	49.22	10.61	$P < 0.01$
	F	57.61	9.75	

M vs. F, *t* tests comparing male vs. female subjects; SCL90R, Symptom Check List 90 Revised.

characteristics of all patients are shown in Table 1. There were no significant differences between the sex groups in age or IBS symptom severity ratings for the 6 months or 24 hours before the first study session. General psychological state at the time of screening was assessed from the General Severity Index of the SCL90-R psychological questionnaire.[21] Female subjects had a higher level of psychological symptoms than male subjects, although the means for both groups were below the clinical cutoff t-score of 63 (see Table 1).

Design

Following a screening visit, subjects were studied during a single session. Intensity ratings of the abdominal pain experienced during the past 24 hours were obtained at the time of the PET study.

Procedure

Before the PET study, using a procedure previously described,[19] a balloon was inserted into the sigmoid colon and a separate balloon into the rectal colon during an endoscopy without premedication. The double-balloon catheter consisted of 2 identical latex balloons (external diameter, 5 cm; length of each balloon, 9 cm) attached to a Silastic elastomer tube (external diameter, 18F) at both the proximal and distal ends (MAK-LA, Los Angeles, CA). The proximal balloon was 40 cm from the anal orifice. After insertion of the catheter, the subject rested for approximately 30 minutes before being transported from the gastrointestinal facility to the PET center. During the study, balloons were inflated at a rapid volume rate (870 mL/min) and held at a constant pressure plateau by a computer-driven pump (barostat).

PET Procedure

The subjects were scanned using a PET scanner (Siemens/CTI 953 tomograph; Siemens-Computer Technology, Inc., Knoxville, TN) collecting 31 contiguous data planes corresponding to an axial depth of 3.375 mm each in a 128 × 128 image matrix. Each subject was positioned in the scanner so that the axis of the scanner was approximately parallel to the glabellar-inion line. An automatic procedure outlined the scalp, and the well-known bulk attenuation coefficient was used to correct the emission scans.

rCBF in each subject was measured 6 times during the session by recording the distribution of cerebral radioactivity following intravenous bolus infusion of the freely diffusible positron-emitting ^{15}O-labeled tracer $H_2{}^{15}O$. For each measurement, individuals received a 25-mCi bolus of $H_2{}^{15}O$. A 120-second scan commenced after the start of the bolus and coincided with the start of the rectal distention where appropriate. Allowing 12 minutes between each injection permitted the decay of background radiation to <10% of the recorded peak. The transmission scan and 6 rCBF measurements were completed in 1.5 hours. During an initial baseline scan, the subjects were asked to remain still with their eyes closed. During the distention scan (60 seconds, 45–mm Hg pressure to the rectum), the subjects were warned that a stimulus would be delivered and that it would be experienced as stool and/or discomfort. Before the anticipation scan, the subjects were informed that the next scan would also involve inflation and that this inflation would be "significantly more intense than the previous inflation." No inflation was actually delivered. The subject was then informed that there would be a break in the scanning procedure while the sigmoid distention took place. The patient remained in the scanner during the sigmoid distentions, which lasted for 15 minutes. Afterward, the resting, rectal distention, and anticipation scans were repeated.

Eight minutes after each injection, subjects were asked to rate their recalled perception of the visceral stimulus. Ratings were made on a validated 20-cm descriptor-anchored visual analogue scale.[22] These ratings were done for qualitative purposes to compare the judgments of the rectal inflation conditions. Due to the delay in making ratings and the small number of inflations, the ratings are not considered sensitive measures for testing sex-related differences in perception of the 45–mm Hg inflation. Also, given the delay, they are clearly not valid measures of the anticipation condition because, by the time the ratings were made, subjects were aware that an intense inflation was not delivered on that trial. Validated semantic differential scales[23] of current mood (arousal, fatigue, anxiety, stress, and attention) were also filled out at 3 time points (before balloon placement and after each anticipation condition).

To ensure consistency, all instructions were delivered using a previously recorded tape. The scans were recorded in the same order for each subject. By necessity, the sigmoid sensitization conditioning had to occur in the middle of the procedure and the anticipation scans were required to follow the rectal distention.

Data Analysis

For each subject, the raw scan data were processed with the following procedures using SPM99 (Wellcome Trust Centre for the Study of Cognitive Neurology) as described in detail elsewhere.[24,25] Head movement between scans was corrected by aligning all scans with the first scan. Each realigned set of scans per subject was registered into the standardized anatomic space of the average magnetic resonance image provided by the

Table 2. Sex Effects on *A Priori* Regions of Interest in Session 1

Region of interest (search volume *x, y, z*)	45–mm Hg: baseline cluster interaction no. voxels/*P*	M	F	Anticipation: baseline cluster interaction no. voxels/*P*	M	F
Female > male						
Left amygdala (12 × 12 × 12 box −24, −6, −16)	167/0.005	D[a]	A[b]	60/0.05	D[a]	
Right amygdala (12 × 12 × 12 box −24, −6, −16)				105/0.02	D[b]	A[c]
Left anterior cingulate (12 × 24 × 20 box 6, 30, 22)	22/0.34		A[b]			
Right anterior cingulate (12 × 24 × 20 box 6, 30, 22)	188/0.03	D[c]	A[a]	218/0.02	D[b]	A[c]
Left midcingulate (8 × 40 × 12 box 4, −2, 36)				179/0.03	D[c]	A[a]
Male > female						
Dorsal pons/PAG (24 × 20 × 10 box 0, −28, −10)	278/0.007	A[a]		162/0.03	A[c]	D[a]
Left midposterior insula (8-mm sphere 44, −4, 2)	47/0.08	A[b]				
Right midposterior insula (8-mm sphere 44, −4, 2)	7/0.25	A[a]				
Right thalamus (14-mm sphere 12, −18, 8)	201/0.08	A[c]	D[c]			

M, males; F, females; A, activations; D, deactivations.
Corrected *P* levels: [a]$0.05 < P < 0.1$; [b]< 0.05; [c]> 0.1.

Montreal Neurological Institute (MNI space). To increase the signal-to-noise ratio and to accommodate variability in functional anatomy, each image was smoothed in x, y, and z dimensions with a Gaussian filter of 12 mm (full width at half maximum).

At each voxel, a model was fit that regressed rCBF on the nuisance effects of global activity and scan order within subjects. For each question of interest, we first used a region-of-interest (ROI) approach to assess sex-by-condition interactions for 7 a priori bilateral brain areas and an additional midline brainstem area (dorsal pons/PAG) that have been previously associated with visceral stimulation responses or with the EMS (see Table 2). The location and size of each ROI was selected according to functional neuroanatomy. However, the shape of the ROIs was either a sphere or a rectangular box (SPM99 small volume correction). To more effectively sample the 2 large structures most reliably associated with visceral pain and to differentiate functionally dissociated subregions, we drew 2 ROIs in the insula (anterior and midposterior) and 3 in the cingulate gyrus (infragenual, anterior cingulate, and midcingulate). A fixed-effects analysis was used to produce contrast images representing changes in brain activity due to differences between conditions for each subject. These contrast images were then entered into random-effects analyses (one image per subject) to assess sex-by-condition interactions ($\alpha = 0.05$). Random-effects analyses are conservative approaches to quantification of brain images. Although generally requiring larger effects than fixed-effects analyses, they allow generalization to the population from which the samples are drawn. Results are graphically presented as statistical parametric maps. In the display, voxels with sex-related differences of $P < 0.05$ (uncorrected) are colored. Statistical results for both the 45–mm Hg inflation and the anticipation condition are presented in Table 2 for each ROI. The *P* values are based on the criterion of spatial extent after correction for total search volume. To interpret these sex-by-condition interactions, condition comparisons were also assessed for male and female patients separately (indicated in Table 2 as activations or deactivations relative to baseline). To assess if any sex differences found were due to group differences in psychological distress, the ROI analyses were repeated using the General Severity Index score from the SCL90-R as a covariate.

After the ROI analysis, each contrast was also tested using a whole-brain approach. This allows preliminary identification of sex-related differences in unsuspected brain regions if the activated clusters are large enough to attain spatial extent significance of $P < 0.05$ after correction for total brain voxels.

Sex-Related Effects Tested

Sex differences in rCBF response during rectal balloon distention and during anticipation of undelivered rectal balloon inflation were tested as follows. The response to the 2 baseline scans was subtracted from the response to the 2 rectal balloon inflations to assess sex-related differences during rectal distentions (45 mm Hg). Identical analyses were performed on the 2 anticipation scans relative to the baseline scans. Analysis of the effects of the sigmoid stimulation is beyond the scope of this report and will be reported elsewhere.

Results

Analysis of Condition by Sex

Gut-directed stimulus: 45–mm Hg rectal distention. As shown in Table 2 and Figure 2 (shown in radiologic orientation), there was a significant sex difference in the dorsal pons/PAG. In addition, there were trends toward differences in the left insula and right thalamus ($P = 0.08$) due to greater activation in male patients compared with female patients. There were also significant sex-related differences in the left amygdala and right ACC resulting from activation in female patients only. Female patients also showed significant activation of the infragenual cingulate cortex, whereas male patients did not. However, the sex interaction was not significant ($P = 0.14$).

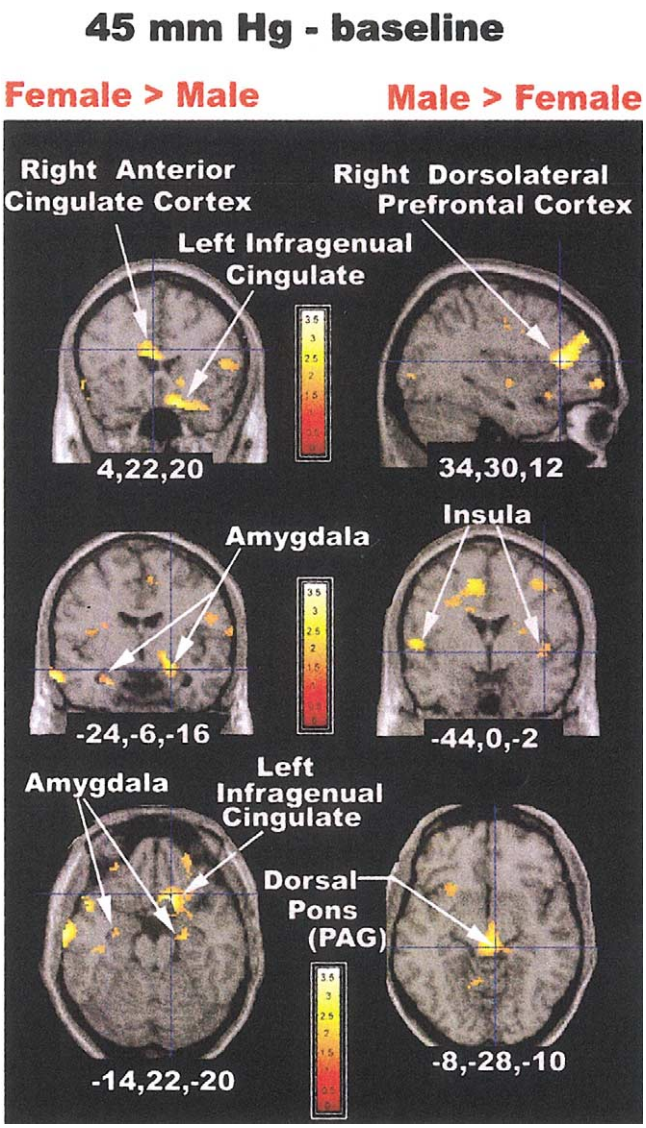

Figure 2. Sex-related differences in the response to the gut-directed stimulus. The response to the 45–mm Hg stimulus for the male and female subjects (compared with baseline) is shown. The *left column* shows voxels of significantly greater activation ($P < 0.05$) in female patients, and the *right column* shows voxels with significantly greater activation in male patients (shown in radiologic orientation).

Central nervous system–directed stimulus: anticipation of undelivered visceral stimulus. Similar to the 45–mm Hg condition, there was a significant sex-related difference in activation of the dorsal pons/PAG resulting from activation in male patients ($P < 0.1$) and a nonsignificant deactivation in female patients. Also, similar to the actual distention, significant sex-related effects in the amygdala reflected activation in female patients ($P < 0.1$) and deactivation in male patients.

Covariate analysis. The ROI analyses were repeated with the General Severity Index score as a covariate of no interest. Overall, the pattern of results was unchanged. All of the significant group differences during the 45–mm Hg stimulus remained significant. Of the regions showing group differences during anticipation, all remained significant with the exception of the right ACC ($P = 0.2$) and the left midcingulate ($P > 0.1$).

Whole-Brain Analysis

In a secondary whole-brain analysis, we examined the same random-effects contrasts correcting for total brain voxels. Clusters of activated voxels large enough to reach significance of $P < 0.05$ after this correction are interpretable without prior regional hypotheses; this analysis therefore represents a conservative approach to preliminary identification of sex-related differences outside of our a priori ROIs.

Gut-directed stimulus: 45–mm Hg rectal distention. Sex affected a large cluster primarily located in the right dorsolateral prefrontal cortex (DLPFC) (corrected $P = 0.06$) extending from BA 46 to BA 9 (peak voxel: 34, 30, 10). Individual group analyses showed activation in male patients and deactivation in female patients ($P < 0.1$). Sex differences also affected a cluster in the left ventromedial PFC (BA 11; corrected $P = 0.04$) bordering on the infragenual cingulate cortex (peak voxel: −24, 45, 3). Female patients showed activation in this area, but there was no change in male patients.

Central nervous system–directed stimulus: anticipation of undelivered visceral stimulus. Sex-related effects were found in 2 clusters. One was located in the right ACC (BA 24/32, peak voxel: 10, 38, 8; corrected $P = 0.03$) and the other in the area of the right middle and inferior temporal gyri (BA 20/21; peak voxel: 40, −8, −24; corrected $P = 0.02$) extending toward the parahippocampal gyrus. The individual group analyses indicated that these interactions were due to significant female activation in the ACC ($P = 0.02$) and a male deactivation ($P = 0.09$) in the region of the middle temporal gyrus (BA 21).

Subjective Ratings

Table 3 shows the perceptual ratings associated with each scan condition. A 2-way mixed-effect analysis of variance (6 stimulus conditions × 2 groups) indicated a main effect of condition ($P < 0.001$) but no significant differences between the sex groups or interaction of sex with condition. The condition effect was due to greater ratings for the 45–mm Hg stimulus compared with the other conditions. Changes in semantic differential scales were also analyzed by 2-way analysis of variance (3 time points × 2 groups). In both groups, fatigue increased over the study while arousal, anxiety, and stress decreased (all $P < 0.01$). The male patients had significantly

Table 3. The Mean Sensory Ratings (20-cm Scale) and *SD* Associated With the 6 Scan Conditions for Day 1

Group	Baseline	p45	Anticipation	Postbaseline	Post-p45	Postanticipation
Males	3.4 (4.1)	11.9 (3.2)	3.8 (4.1)	4.2 (3.8)	11.3 (4.8)	5.1 (5.2)
Females	2.7 (2.8)	12.3 (3.0)	3.2 (3.6)	4.6 (4.7)	11.1 (3.3)	2.7 (3.3)

NOTE. SD appear in parentheses.

higher arousal ratings ($P < 0.05$) and lower fatigue ratings ($P < 0.05$) over the course of the study compared with the female patients, indicating overall greater arousal during the study in the male patients.

Discussion

Despite considerable overlap, male and female patients with IBS showed fundamental differences in their brain response to a visceral stimulus as well as to a signaled but undelivered stimulus. As shown in Figure 1, the most consistent differences were in brain regions concerned with processing of pelvic visceral afferent information (dorsal pons/PAG, insula) as well as in EMS circuits (amygdala) and cortical regions modulating the EMS (ACC subregions, PFC).

Most functional brain imaging studies assessing the rCBF response to somatic[26] and visceral[27–34] stimulation indicate consistent activation in the anterior insula and ACC, with noticeable variation in the reported subregion of cingulate activity.[35] Despite considerable overlap, sex-related differences in rCBF responses during somatic[36] and visceral[13,14] stimuli have been reported. Whereas Berman et al.[13] reported greater insula activation by aversive rectal stimulation in male subjects with IBS, Kern et al.[14] reported that healthy female controls had greater insular and ACC activation compared with males and overall greater cortical volume activation. Paulson et al. also found greater female activation in the insula and PFC using noxious cutaneous heat stimuli in healthy subjects.[36] Because different paradigms were used in each of these studies, a direct comparison of results is not possible.

In principle, sex-related differences in activation patterns could arise from either a sex-related difference in peripheral encoding and/or processing of pelvic visceral afferent input by the brain and/or from a greater recruitment of stress circuits by the psychological components of the distention paradigm (anxiety, fear, and so on). In an attempt to differentiate between these possibilities, we will first discuss sex-related differences observed during the gut-directed stimulus only (visceral afferent stimulation) and then compare these results with those observed during both the actual and anticipation conditions.

Sex-Related Differences Only Seen During Visceral Afferent Stimulation

Brain regions of greater male activation. We have previously shown in 2 independent patient samples a bilateral increased mid-insula activation in male patients compared with female patients with IBS.[13] We have also reported that insula activation is seen with rectal distention but not during anticipation of such distention.[33] In the current study, in a much larger sample, we confirm both findings; during moderate distention of the rectum (but not during the anticipation condition), male patients showed greater activation in the insula (activation in male patients and no significant activation in female patients). The insula receives input from spinothalamic lamina I neurons and has been considered the visceral sensory cortex.[37] It participates in many other functions,[38] including emotional,[39] and visceromotor responses.[40] Based on retrograde viral labeling studies in the rat, the insular cortex is involved in cortical regulation of cardiosympathetic, sympathoadrenal, and, to a lesser degree, celiac sympathetic regulation.[40] Even though autonomic responses were not measured in this study, the greater male activation of the insular cortex is consistent with the reported greater male cardiosympathetic response to rectosigmoid inflations.[41]

Another brain region that showed sex-related differences (activation in male patients and deactivation in female patients) only in response to visceral stimulation was the right DLPFC (BA 46, 9). DLPFC is consistently activated when verbal or visuospatial information is maintained in working memory and actively processed, and there seems to be right lateral specialization for visuospatial working memory.[42] Activation of DLPFC has been reported in somatic and visceral pain studies.[26] In contrast to medial PFC, intense emotions (anxiety or depression) are associated with suppression of rCBF in the DLPFC.[43] Deactivation of predominantly right DLPFC has been reported consistently during memory-driven emotions in healthy subjects and patients with posttraumatic stress disorder, including victims of sexual abuse.[44] It has been suggested that deactivation of DLPFC is mediated by direct and indirect projections from limbic structures, in particular the infragenual cingulate cortex.[45] The greater male responses in DLPFC may

therefore reflect decreased affective and greater cognitive activity during the visceral stressor compared with females.

Brain regions of greater female activation. During rectal distention, female patients showed greater activation in the right ACC (BA 24, 32), infragenual cingulate (BA 25), and adjacent ventromedial PFC (BA 11), all regions frequently activated by emotional stimuli.[42,44] The difference was a result of activation in female patients and deactivation (ACC) or no activation (infragenual, ventromedial PFC) in male patients. The medial PFC and infragenual cingulate receive afferent input from DLPFC (inhibition) as well as from monoaminergic ascending fibers, including those arising from the noradrenergic LC. Both the ACC and ventromedial PFC have reciprocal projections to the infragenual cingulate. Most projections from the infragenual cingulate (ventral pathway) go to the thalamus, hypothalamus, insula, and extended amygdala (including bed nucleus of the stria terminalis), with some projections going to nuclei within the dorsal pons.[46] This pathway also provides descending projections to autonomic cell groups of the brainstem, PAG, parabrachial nucleus, dorsal vagal complex, and intermediolateral column of the spinal cord.[47] Consistent with these projections is the role of the infragenual cortex in the modulation of cardiovascular and splanchnic sympathetic function.[48] Thus, the greater activation of the infragenual cingulate is consistent with the hypothesis of a sex-related difference in both affective as well as autonomic response to the pelvic visceral stimulus.

These findings suggest that male and female patients with IBS have a fundamental difference in brain response to aversive pelvic visceral stimuli. Male patients with IBS show greater activation of regions involved in nociceptive and cognitive processing, motor planning, and sympathetic responses. Consistent with this explanation is the reported finding of greater male cardiosympathetic and sympathoadrenal responses to stressors[49] and visceral[41] pain as well as our current findings that male patients reported greater arousal during the experimental paradigm. On the other hand, the lack of deactivation (right DLPFC) of cognitive brain regions in female patients and simultaneous activation of limbic areas (ACC, infragenual cingulate, ventromedial PFC) is consistent with the reported pattern observed during strong emotional stimuli, resulting in suppression of cognitive processes and switching to an automatic emotional response.[42]

Sex-Related Differences Seen in Both the Visceral Afferent Stimulation and the Anticipation Conditions

Brain regions identified during both types of conditions include brain regions that receive input from both pelvic visceral afferents as well as from supraspinal circuits of the EMS.

Brain regions of greater male activation. During both conditions, significant sex-related differences (activation in male patients and little activation in female patients) were observed in the dorsal pons/PAG, a region including the Barrington's nucleus, LC complex, parabrachial nucleus, PAG, and raphe nuclei.[46] These brain regions, which frequently show reciprocal connections by corticotropin-releasing factor–positive projections and are likely to be coactivated during visceral stimulation, play an important role in the processing of pelvic visceral information (Barrington's nucleus, LC, parabrachial nucleus),[46] in reflex regulation of hindgut responses (Barrington's nucleus), and in integrated autonomic and antinociceptive responses to psychological and physical stressors (PAG).[50] Even though spatial differentiation between these small adjacent structures is not feasible with PET, one may speculate about possible mechanisms underlying the observed sex-related difference in activation of the dorsal pons/PAG region. (1) For example, Barrington's nucleus receives afferent input from several sexually dimorphic regions, including the medial preoptic nucleus, the bed nucleus of the stria terminalis, the lateral hypothalamus, and dimorphic cortical regions.[46,51] (2) Several of these nuclei receive input from the infragenual and insular cortex as well as the paraventricular nucleus of the hypothalamus. Thus, it might be speculated that different descending input from some of these regions (insula, infragenual cingulate) could explain our findings. (3) The LC is the major source of ascending noradrenergic projections,[52] including those to cingulate subregions, PFC, and amygdala. LC is a sexually dimorphic region in both humans and rats.[53–55]

In summary, plausible explanations for the greater activation of the dorsal pons/PAG region in response to both actual and anticipation of visceral stimulation include differences in autonomic and/or antinociceptive responses to pelvic visceral stressors or to their threat. Because repeated stress has been found to result in an up-regulation of corticotropin-releasing factor gene expression in some of the involved nuclei (Barrington's nucleus, LC), the greater responsiveness of this brain region may also be related to sex-related differences in stress sensitization.

Brain regions of greater female activation. During both conditions, a significant sex-related difference (activation in female patients and deactivation in male patients) was observed in the region of the amygdala and the ACC (rostral to midcingulate). Activation of the amygdala complex (including the bed nucleus of the stria

terminalis) occurs primarily during stimuli associated with fear and anxiety[56] but also in response to visceral stimuli.[15,33] Preliminary observations suggest that deactivation of the amygdala during a 60-second visceral stimulation may be observed.[57] Due to the limited spatial resolution of the PET imaging technique, it is not possible to differentiate between different subregions of the extended amygdala complex. Similarly, the limited temporal resolution (activation of the amygdala in response to psychological stress occurs within milliseconds to seconds) does not allow us to determine if the sex-related differences may be due to differences in amplitude or in the time course of changes in blood flow. For example, it may be speculated that whereas male patients may show greater initial activation, inactivation and inhibition may occur more rapidly than in female patients. The fact that similar sex-related differences were seen under both stress conditions and that they were mirrored by differences in dorsal pons/PAG activation suggest that differences are related to circuits involving reciprocal connections between pontine nuclei, such as the LC complex, and the extended amygdala, which also involve ACC and medial PFC.

Activation of ACC has been reported to correlate with subjective ratings of unpleasantness of both somatic[58] as well as visceral[30,32] stimuli, and activation of this ACC subregion has been reported in several studies using visceral stimuli.[14,29,34,35,59] ACC receives afferent input from ascending monoaminergic projections, including those from the LC, and has reciprocal connections with the infragenual cingulate and ventromedial PFC, which in turn project to the amygdala.[60] Thus, the greater activation of ACC in female patients may be related to the greater activation of the amygdala.

Limitations

Several limitations of the current study should be mentioned. First, although matched for age and symptom severity, the male and female groups differed on global distress (despite both groups falling in the normal range). However, the covariate analysis suggests that the observed sex differences in regional brain activation are unlikely to be primarily a result of differences in global distress. Hormonal fluctuations as part of the normal menstrual cycle may alter pain and emotional responding in women. Phase of the menstrual cycle was not assessed in the female sample studied, so it is not possible to rule out hormonal variables as contributing to the differences seen. The anticipation condition used in this study was not based on a multiple trial conditioning paradigm, and it was not possible to directly assess the strength of the anticipation response because the stimulus assessments were performed well after the anticipation period (and clearly after subjects knew they had not received an intense rectal stimulus). However, similar to previous studies using a similar anticipation condition[33,61] and a recent study using a conditioning paradigm,[62] we found multiple areas of common activation between the actual and anticipated visceral stimulus. In addition, there is evidence from somatic pain studies that verbal warnings regarding a possible aversive stimulus (e.g., electric shock) without actual experience of the stimulus led to stress-induced hyperalgesia[63] due to anticipatory anxiety. Thus, although it is not possible to determine if the results are due to single trial conditioning or simply expectation based on the verbal instructions, the anticipation condition did lead to a significant central response compared with rest. Finally, it should be noted that some of the significant sex interactions are in part due to deactivations in one group and activations in the other. Although deactivations in functional imaging studies are commonly reported, their physiologic relevance continues to be a subject of discussion.[64]

Summary and Conclusions

These results are consistent with fundamental differences between male and female patients with IBS in their brain response to a visceral stimulus and a condition in which the same stimulus is signaled but not delivered (Figure 1). The most consistent differences seen during both conditions are in brain regions concerned with processing of pelvic visceral afferent information (dorsal pons/PAG, insula) as well as in EMS circuits (amygdala) and cortical regions modulating EMS output (ACC subregions, ventromedial PFC). In general, male patients show greater activation of cognitive areas (DLPFC), central sympathetic areas (insula, PAG), and inhibition of limbic regions, whereas female patients show greater activation of affective and autonomic regions (ventromedial PFC, infragenual cingulate, amygdala). These differences may explain reported sex-related differences in emotional, autonomic, and antinociceptive responses between male and female patients with IBS and may be related to reported differences in treatment responses to medication.

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