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Sex Differences in Emotion-related Cognitive Processes in Irritable Bowel Syndrome and Healthy Control Subjects

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Abstract

Background/Aims—Greater responsiveness of emotional arousal circuits in relation to delivered visceral pain has been implicated as underlying central pain amplification in Irritable Bowel Syndrome (IBS), with females showing greater responses than males.

Methods—Functional MRI was used to measure neural responses to an emotion recognition paradigm, using faces expressing negative emotions (fear and anger). Sex and disease differences in the connectivity of affective and modulatory cortical circuits were studied in 47 IBS (27 premenopausal females) and 67 healthy controls (HCs; 38 premenopausal females).

Results—Male subjects (IBS+HCs) showed greater overall brain responses to stimuli than female subjects in prefrontal cortex, insula, and amygdala. Effective connectivity analyses identified major sex and disease related differences in the functioning of brain networks related to prefrontal regions, cingulate, insula, and amygdala. Males had stronger connectivity between anterior cingulate subregions, amygdala, and insula, whereas females had stronger connectivity to and from the prefrontal modulatory regions (medial/dorsolateral cortex).

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Conclusions—Male IBS demonstrate greater engagement of cortical and affect related brain circuitry compared to male controls and females, when viewing faces depicting emotions previously shown to elicit greater behavioral and brain responses in male subjects.

Keywords

Irritable bowel syndrome (IBS); sex differences; emotion recognition

1.0.0 INTRODUCTION

Sex-related differences in the structure and function of the human brain [36] have paralleled sex-specific prevalence rates of chronic pain disorders [19,47,54]. Sex based differences in the brain's response to symptom related affective and cognitive stimuli may be important for understanding the pathophysiology of these disorders – in terms of increased susceptibility to develop these disorders, and in order to develop more effective individualized therapies [67].

Irritable Bowel Syndrome (IBS) occurs with a slightly greater prevalence in females [10,19,43,50], and sex related differences in visceral perception, autonomic nervous system and brain responses to visceral stimuli have been reported [11,39]. Male subjects show greater sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) axis responses to certain types of stress, whereas females show reduced vagal tone and greater visceral hypersensitivity [11,70]. Brain imaging studies in rodents [78] and in IBS subjects [44–46,73,77] during aversive visceral stimulation, and expectation of such stimuli, demonstrate greater engagement of cortical regions (insula [INS] and dorsal prefrontal cortex [PFC]) in males, and greater engagement of affective brain regions and related circuits in females (amygdala [AMYG], subgenual cingulate cortex [sgACC]) [40,53]. These findings suggest that in response to gut (and disease) related stimuli, IBS subjects show sex-related differences in brain activation and functional connectivity.

In response to emotion-related stimuli (including faces, images, words, odors, music) female subjects generally show greater brain activation related to emotions of sadness, disgust and unpleasantness [67], whereas men demonstrate greater neural responses to emotions such as anger, fear, and guilt [29,67]. Differences in brain responses to the viewing of faces expressing different emotions have been used to measure differences in the engagement of emotion-related brain circuits and their cortical modulation [13,20,22,58,66], as well as disease [18,56,63,66,76] and sex-related [21,29,36,67] differences in these circuits. Even though differences in amygdala responsiveness and cortical modulation of such responses when viewing fear related faces have been demonstrated, this paradigm is not associated with changes in subjective emotions or autonomic responses [15,23].

In the current study, we used the paradigm of viewing of negative affective (fear and anger) and neutral faces to test sex and IBS related differences in brain response associated with cognitive processes, i.e. processing negative emotions. We studied a large sample of male and predominantly premenopausal female IBS subjects and matched HCs by monitoring brain responses to negative facial emotions (fear and anger) in the NimStim paradigm [71], which is a variation on the Ekman faces [16,71]. We aimed to test the following hypotheses:

1) Greater brain responses in affective regions, and less recruitment of prefrontal inhibitory regions will be observed in IBS compared to HCs, in male compared to female subjects, in male IBS compared to female IBS, and in male HCs compared to female HCs. 2) Greater regional brain activation by the emotional faces paradigm will be accompanied by changes in the effective connectivity of the emotion related circuit and its cortical modulatory input.

2.0.0 MATERIALS AND METHODS

2.1.0 Participants

This study was approved by the institutional review board of the University of California, Los Angeles. All subjects provided written informed consent to participate. IBS subjects were recruited through the UCLA Digestive Disease Clinic and from community advertisements. The diagnosis of IBS was confirmed using Rome III [14] criteria during a clinical examination by a gastroenterologist or nurse practitioner experienced in functional GI disorders. IBS is defined as recurrent abdominal pain or discomfort for at least 3days/month in the last 3 months and is associated with two or more of the following: 1) Improvement in defecation. 2) Onset associated with a change in frequency of stool. 3) Onset associated with a change in form (appearance) of stool. Healthy control subjects (HCs) were recruited by advertisement and screened via medical exam for absence of functional pain disorders. Inclusion criteria for all subjects included the absence of current or past psychiatric illness or substance abuse disorder and the absence of major medical or neurological conditions. No subjects were taking medications for 30 days prior to scanning. 14 healthy controls from another onsite imaging study took a placebo medication on the day of scanning. In order to determine if the HC subjects taking placebo could be combined with the HC subjects not taking placebo, an independent sample t-test was applied using Statistical Parametric Mapping V8 [51] and indicated no statistically significant differences in brain response to stimuli on the faces paradigm between the two groups, supporting the combination of the two groups to create a larger sample size of HC subjects.

2.2.0 Questionnaires

Subjects completed the UCLA Bowel Symptom Questionnaire (BSQ) [49] to measure symptom severity, and anxiety was measured using the HAD Anxiety measure and depression was measured using the HAD Depression [81]. For female subjects, menopause status was assessed by a self-report question, which categorized the subjects as either premenopausal or postmenopausal, and the majority of the studies were done during the follicular phase of the menstrual cycle. The majority of female subjects in the study (82%) were not taking any oral contraceptives.

2.3.0 fMRI Data Acquisition

fMRI was performed using a 3.0T MRI scanner (Siemens Trio; Siemens, Erlangen, Germany). A high resolution structural image was acquired from each subject with a magnetization-prepared rapid acquisition gradient-echo (MP-RAGE) sequence, repetition time (TR) = 2300 ms, echo time (TE) = 2.85 ms, 256 slices, 160*240 matrix, 3 mm voxel size. Functional blood oxygen-level dependent (BOLD) images were acquired (TR = 3000 ms, TE = 28 ms, flip angle = 90°, 38 slices, slice thickness = 3 mm) while subjects

completed two runs of the emotional faces tasks. Stimuli were presented via MRI-compatible goggles using Superlab 4.0 software (Cedrus Corp, San Pedro, CA). Subjects responded using an MRI-compatible button box by pressing one of two buttons with the right hand.

2.4.0. fMRI Imaging Task: Faces Paradigm

One of the most commonly used experimental paradigms for fMRI studies has been the viewing of images with negatively valenced facial expressions [24,27,31]. Paradigms using negative emotional facial expressions have also been used in several imaging genetics studies demonstrating increased hyperresponsiveness of emotion related networks (including the amygdala) in healthy control subjects with increased harm avoidance and SERT gene polymorphisms [25,26,34], and within several psychiatric disorders including posttraumatic stress disorder [18,63], autism [76], trait anxiety [56,66], and Parkinson's disease [61].

During this fMRI study, brain responses to the NimStim Emotional Faces Viewing task [71] were measured. During matching emotions (ME), subjects viewed a target face depicting an angry or fearful expression and were asked to select one of two other faces that expressed the same emotion. During matching form (MF), a condition controlling for the sensory-motor aspects of the ME task, subjects viewed a target circular shape (approximately the same size as a human face) and were asked to select one of two other shapes that best matched the target. Participants also viewed the same target faces as in the matching condition, but had to judge which of two linguistic labels, such as angry or afraid, best described the emotion (identifying emotion (IDE)). As a control task, the subjects viewed the same target faces, but labeled the faces based on their gender, either male or female, and not on affect (identifying gender (IDG)). We did not analyze the results of the linguistic labeling task in this manuscript. Stimuli were shown with randomized sequences counter-balanced across 2 runs. Each condition was presented as a block of 6 images, with each image presented for 3 sec, with a total block length of 18 sec. In each run, each condition (i.e., match forms) was randomly presented. An instruction cue was presented for 3 sec prior to each block and a rest period of 6 sec followed each block. Each run began with a 30 second anticipatory baseline.

2.5.0 Data Analysis: Image Processing and Data Analysis

Preprocessing—The first two volumes were discarded to allow for stabilization of the magnetic field. The remaining functional images were slice-time and motion corrected, spatially normalized to the MNI template, and spatially smoothed with an 8 mm^3 Gaussian kernel using SPM8 (Welcome Department of Cognitive Neurology, London, UK). Brain activity during the Emotional Faces Viewing task was estimated for each subject with first-level fixed effects general linear models specifying the 30 second anticipatory baseline (from 2 runs, 3sec inter-stimulus intervals, 3sec cues, ME, MF, IDE, and IDG. Brain activity was measured by contrasting brain responses during ME and MF tasks.

Statistical Parametric Mapping—Sex differences during the matching tasks (ME-MF) were tested in apriori specified regions comprising emotional arousal, cortico-modulatory and homeostatic afferent circuitry by applying a second-level random effects general linear

model specifying group (Male IBS, Female IBS, Male HC, and Female HC) as a factor via the full factorial model option in SPM8. Here the group factor represents the interaction of disease (IBS, HC) and sex (male, female). After thresholding whole brain activity maps at $p<.005$ uncorrected, each region of interest was tested using linear contrast analysis and small volume correction in SPM which corrects for the number of voxels comprising each region of interest. Furthermore, we applied false discovery rate (FDR) to correct the voxel-corrected probability values to control for the number of ROIs tested. Cluster level significance was considered at $q<.05$ [3,4,28].

Linear contrasts—For each region of interest we specified contrasts to test the main effects of disease (IBS vs. HC) and sex (Males vs. Females). Furthermore, we explicitly tested two global (omnibus) interaction contrasts to determine whether differences in sex depend upon disease, e.g., [HC(Male-Female) - IBS(Male-Female)] and whether disease differences depend on sex [Female(HC-IBS) - Male(HC-IBS)]. We also tested four a priori linear contrasts: 1) Are there sex differences in brain activity within IBS? (Male IBS vs. Female IBS), 2) Are differences in brain activity in males due to disease? (Male IBS vs. Male HC), and 3) Are differences in brain activity in females due to disease (Female IBS vs. Female HC), 4) Are there sex differences in brain activity within HC? (Male HC vs. Female HC), To examine whether anxiety and depression contribute to group differences, we calculated two additional models as described above entering anxiety and depression as a covariate.

Regions of Interest—Based on prior research, sixteen regions of interest (ROIs) were chosen a priori and consisted of emotion related brain areas including amygdala (AMYG), cingulate cortex subregions (anterior mid cingulate cortex [aMCC], subgenual cingulate cortex [sgACC], pregenual cingulate cortex [pACC], posterior cingulate cortex [PCC], anterior insula [aINS], hippocampus [HIPP], hypothalamus [HYPO], dorsal pons [PAG], and nucleus accumbens [NACC]) [37,38,59] as well prefrontal regions involved in cognitive control processes during detection and regulation of affectively salient stimuli (ventrolateral prefrontal cortex [vlPFC], ventromedial prefrontal cortex [vmPFC], dorsal medial prefrontal cortex [dmPFC], and dorsolateral prefrontal cortex [dlPFC]) [57,60]. We also examined the involvement of the posterior insula (pINS) as it provides interoceptive signals to the anterior insula (aINS) and mid insula (mINS) for integration with cortical and affective signals to provide the basis for feeling and self [12].

Effective connectivity analysis was applied to test for hypothesized group differences (IBS vs. HC, Male vs. Female, Male IBS vs. Female IBS) in the engagement of a stress-related emotional arousal circuits during the viewing of specific negative emotional faces (anger and fear). Described previously [38,59,65], the affective network is characterized by core inhibitory circuitry comprising pACC/vmPFC, AMYG and sgACC in addition to extended connectivity/interactions with the HIPP, aMCC, aINS, mINS, pINS, dlPFC, and vlPFC. In line with known anatomical connectivity in the macaque [74], a priori connections between nodes of the network were specified to test for differences in the effective connectivity of the a) the core inhibitory circuit (pACC/vmPFC to AMYG) b) the influence of top-down cognitive control influences (dlPFC, vlPFC) and extended affective system connectivity

with the aMCC, PCC, HIPP, and INS. Given the limitations in specifying all potential bilateral connections we specified bidirectional AMYG connectivity only (Figure 1).

For each region comprising the nodes of the circuit, brain activity was extracted from the spatial location of the peak voxels identified from the results of the primary SPM analyses. After specifying the structural model, path analysis using a structural equation modeling (SEM) framework was performed with Amos 18.0 conducting full information likelihood estimation. Standard errors for parameter estimates (unstandardized betas) were obtained via 500 bootstrapped samples and used to calculate bias-corrected 95% confidence intervals based on the normal distribution. Residual variances, representing external input into the system (e.g. unspecified regions, psychological characteristics), were fixed at 35% [48] of the observed regional variances within group (IBS, HC) and sex (Male, Female) conditions. Group differences (Males IBS, Female IBS, Male HC, Female HC) in the effective connectivity of the cortico-limbic network in IBS and HCs were tested using specific multi-group tests for invariance [32,48]. Given our hypotheses, we specified three a priori contrasts examining sex and disease effects: 1) Male IBS vs. Female IBS, 2) Male IBS vs. Male HC, and 3) Female IBS vs. Female HC. Critical values for the multigroup significance tests were based on a one degree of freedom chi-square distribution where critical values where $\chi^2\Delta=3.84$, p=.05 and $\chi^2\Delta= 6.64$, p=.01. However, false discovery rate (FDR) was applied to control for the number of paths tested per group comparison (n=19) and significance was considered at q<.05 [3,4,28]. For interpretation of results, unstandardized betas, which represent effect size in terms of standard deviation units, were interpreted as weak (β=0 to.30), moderate (β=30 to.80), or strong (β= >.80).

Data analysis of non-imaging data—Differences in clinical and demographics variables were examined using the general linear model in SPSS version 19 by specifying a 2×2 independent group ANOVA.

3.0.0 Results

3.1.0 Clinical and behavioral characteristics

Table 1 summarizes clinical and personality characteristics of the four study groups (Male IBS, Female IBS, Male HC, Female HC). Although mostly within normal clinical ranges, 18% of all subjects (IBS+HCs) had anxiety scores above the clinical cutoff and <1% of all subjects had depression scores above the clinical cutoff. Compared to HCs, IBS subjects as a group showed significantly higher anxiety symptoms (F=14.58, p<0.001), with male IBS subjects showing slightly higher levels than female IBS (F=4.26, p=0.041). Small group differences were also observed for depression symptoms between IBS and HCs (F=21.43, p<0.001) and similarly between males and females (F=6.62, p=0.011). The female sample was predominantly premenopausal (93% of female HCs and 92% of female IBS subjects).

3.2.0 Disease and sex-related differences in brain responses

3.2.1 Disease related differences—No statistically significant differences in the response of emotional arousal regions were found between all IBS subjects (N=47) and all HCs (N=67) while viewing negative emotional faces.

3.2.2 Sex related differences—When comparing all female subjects (IBS+HC; n=65) with all male subjects (IBS+HC; n= 49), significant differences in emotional arousal regions were observed. During the viewing of negative faces, male subjects showed greater activation compared to females in subregions of the PFC (mPFC, left dlPFC), of the cingulate cortex (including PCC, sgACC, and aMCC,) and of the INS (mINS, pINS), as well as the NACC and the HIPP (Table 2, Figure 2a). Female subjects showed greater activity compared to males in the right dlPFC and PAG while viewing negative faces (Figure 2b).

3.2.3 Interaction between sex and disease—Interaction contrasts examining the differences between males and females related to disease [(Male IBS-Female IBS) – (Male HC-Female HC)] indicated greater differences in brain responses at the set level between male and female IBS subjects as opposed to male and female HCs for the response of right amygdala, bilateral insula, bilateral pregenual cingulate cortex, and right mPFC (Table 3, Figure 3). No other interaction effects were observed.

3.2.4 Sex and disease related differences

I. Are there sex differences in brain activity within IBS? As shown in Table 4, male IBS subjects (N=20) compared to female IBS (N=27) showed greater activation in several subregions of the PFC (mPFC, dlPFC), ACC (pACC, sgACC, aMCC), PCC, INS subregions (mINS, pINS), as well as HIPP, HYPO and NACC (Table 4, Figure 4a).

II. Are differences in brain activity in males due to disease? Male IBS subjects compared to male HCs showed greater activation in the AMYG (Table 4, Figure 4b).

III. Are differences in brain activity in females due to disease? No significant differences were seen for female IBS subjects compared to female HCs (Table 4).

IV. Are there sex differences in brain activity within HC? Female HCs compared to male HCs (Table 4, Figure 4c) showed greater activation in the cognitive modulatory regions of the dlPFC and the PAG. Male HCs (N=29) compared to female HCs (N=38) (Table 4, Figure 4d) showed greater activation in several subregions of the ACC (pACC, sgACC), mINS and NACC. For specific within group ROI activity (activations and deactivations) see supplemental Table 1.

When controlling symptoms of anxiety and depression, results remained. In addition male IBS compared to female IBS had greater activity in the HYPO (MNI -10,-2,-2; Z=2.9; k=3; p=0.045) when accounting for anxiety and greater activity in the aINS (MNI -42,0,-4; Z=4.1; k=266, p=0.002) when accounting for depression.

3.3.0 Effective connectivity in the stress related cortical-affective circuitry during the matched emotions versus the matched forms contrast (ME-MF)

Widespread group differences were observed in the connectivity of prefrontal and hippocampal modulatory inputs to AMYG and aINS, as well as the connectivity between INS subregions, and cingulate subregions. The signified chi-square values for the paths

specified in the model survived FDR correction. A complete list of these differences are shown in Tables 5a and 5b, and Figure 5.

3.3.1 Connectivity within the emotional arousal circuit—Connectivity between AMYG and sgACC was weakly positive in males (HCs and IBS) and weakly negative in females (HCs and IBS), but these differences did not reach statistical significance. All groups showed moderate positive connectivity from sgACC to pACC/vmPFC, with female IBS showing the strongest connectivity (β=1.39) which was significantly higher than female HC ($\chi^2\Delta$=11.7, p<.01) and male IBS ($\chi^2\Delta$=13.5, p<.01). Female IBS showed a weak positive connectivity pACC/vmPFC to the AMYG, (β=.24) whereas male IBS subjects had moderate and negative connectivity (β=−.39) resulting in a significant group difference, $\chi^2\Delta$=5.6, p<.05. Additionally, the input from pACC/vmPFC on aINS, showed moderate positive connectivity for IBS as a group with significant greater connectivity observed for female IBS compared to female HC, $\chi^2\Delta$=7.6, p<.01. There was a significantly greater positive connectivity from aMCC to aINS for male IBS (β=.99) compared to female IBS (β=.41) ($\chi^2\Delta$=4.4, p<.01). Weak inputs were observed for all groups from the AMYG to the aINS and moderate positive inputs from the aINS to the AMYG, without any significant group differences. Thus, significant group differences were observed for modulatory influences of anterior aspects of the cingulate cortex with the AMYG and the aINS, with male subjects showing greater positive connectivity between aMCC and aINS and greater moderate negative connectivity from pACC/vmPFC to AMYG.

3.3.2 Prefrontal and hippocampal modulatory inputs to the emotional arousal circuit—Significant group differences were observed for the influence of dlPFC (but not vlPFC) on the pACC/vmPFC. Specifically, female IBS showed a strong moderate positive influence of the dlPFC on the pACC/vmPFC (β=.78). This connectivity was absent in male IBS (β=.03) and weakly negative in HC subjects. As such, significant sex differences were observed with female IBS having greater connectivity between these cortical regions, compared to male IBS ($\chi^2\Delta$=5.9, p<.05) and female HCs ($\chi^2\Delta$=8.2, p<.01).

The HIPP showed a weak negative connectivity to AMYG in the IBS group, whereas connectivity in HCs was positive with male HC showing the strongest connectivity (β=.78) followed by female HC (β=.32). This resulted in significant group differences in lesser engagement of this circuit for male IBS compared to male HC ($\chi^2\Delta$=4.5, p<.05), and lesser for female IBS compared to female HC ($\chi^2\Delta$=3.8, p<.05). Significant differences were also observed for AMYG to HIPP with female IBS having a moderate positive connectivity (β=.64) compared to weak positive connectivity in the other 3 groups (β's <=.16). Statistical significant differences were observed for female IBS greater than female HC ($\chi^2\Delta$=13.0, p<.01) and male IBS ($\chi^2\Delta$=9.3, p<.01). Thus, disease related group differences were observed in the reciprocal connectivity between HIPP and AMYG, with HCs having stronger positive connections than IBS with the male HCs showing the strongest connection; and with IBS having stronger positive connections than HCs in the AMYG to the HIPP, with female IBS showing the strongest connection.

4.0.0 Discussion

The aim of the study was to identify disease and sex related differences in brain responses to an emotion recognition paradigm unrelated to gastrointestinal (GI) stimuli or symptoms. The paradigm was limited to images of negative emotions (anger and fear), which have shown to elicit greater brain responses in males compare to females [8,29]. The main findings of the current study were: 1) Functional activity during the viewing of emotional faces did not differ when IBS subjects as a group were compared to HCs. 2) Male subjects showed greater functional activations in PFC, INS, NACC and HIPP, than female subjects regardless of disease group. 3) Male IBS subjects compared to female IBS showed greater activations in subregions of the PFC, ACC, and INS, as well as the PCC, HIPP, HYPO and NACC. Male IBS subjects showed greater activations in the AMYG compared to male HCs 4) Effective connectivity analyses identified major sex and disease related differences within the emotional arousal circuit, and in the modulatory influences of prefrontal and hippocampal regions on this circuitry. These findings for the first time demonstrate differential brain activity and connectivity of male IBS compared to female IBS and male HCs to a disease unrelated stimulus. Similar to the published greater responsiveness of an emotional arousal circuit to an IBS related stimulus in female IBS [69,72], the current findings in males demonstrate that greater IBS-related engagement of emotional arousal and cognitive modulatory brain circuitry can be demonstrated in male subjects. However, different stimuli are required in male and female subjects to elicit these disease and sex related differences.

4.1.0 Sex and disease related differences in brain activation in response to the emotion recognition task

4.1.1 Group differences between IBS and HCs are sex dependent—We found no statistically significant differences between IBS compared to HCs. These results differ from reports using controlled rectal balloon distension, where female IBS subjects had greater engagement of emotional arousal circuits, male IBS had greater engagement of homeostatic afferent and endogenous pain modulation regions, and HCs showed greater engagement of cognitive modulatory regions [69]. Our findings suggest that the failure to detect a disease-related difference was due to sex-related differences in these responses: When comparing male IBS subjects with male HCs, subjects showed greater amygdala responses to the emotion recognition paradigm, while no such difference was observed between female IBS compared to female HCs.

4.1.2 Greater brain responses in male subjects in viscerosensory, affective and cognitive brain regions—When comparing all males with all females, we found greater activation in mid and posterior subregions of the insula, subregions of the PFC, amygdala, as well as subgenual and posterior cingulate cortical subregions. Male IBS subjects showed similar differences in activating brain regions as the male group as a whole (IBS and HC), but also showed greater activity in HIPP, HYPO and NACC. These findings of greater cortical responses in males are consistent with previous findings using abdominal pain related tasks in both humans [5,17,30] and in rodents [68,79], where greater reactivity was observed in the prefrontal cortex and the mid and posterior insula. However, greater amygdala activity in males has not been reported in previous IBS samples. When viewed

together with previous reports, the current findings suggest a generalized sex and IBS disease difference in brain responses to a variety of emotionally salient stimuli, including threat of pain, aversive visceral stimuli and viewing of negative emotional faces [20,67,75]. One may speculate about the observed greater responsiveness in male subjects to this particular stimulus. Recognition of emotions of anger and fear in a potential adversary can be seen as early correlates of the fight and flight response, a context in which males show greater hypothalamic-pituitary-adrenal (HPA) axis and sympathetic/sacral parasympathetic nervous system responses [2,55,64,80].

4.1.3. Greater responses in female subjects in regions of cortical-pontine circuits—Female HCs showed a greater response than male HCs in the right dlPFC and the PAG. This sex difference was not observed in the IBS group. These findings may be related to a specific, female response to the recognition of certain emotions [33], which is not engaged in IBS.

Group differences based on disease and sex were observed in symptoms of anxiety and depression, with IBS subjects and males as a group (IBS+HCs) showing slightly higher levels on both measures. When anxiety and depression were statistically controlled these differences remained, with the exception of increased activity in male IBS compared to female IBS in the hypothalamus for anxiety and in the anterior insula for depression. This suggests that the majority of the observed differences in the brain's response to the emotion recognition faces task were not explained by the differences in underlying differences in mood and affect, but reflect fundamental differences in the brain's responsiveness to this stimulus.

4.2.0 Sex and disease differences in effective connectivity of brain circuits related to emotional arousal and cortical modulation

4.2.1 Sex related similarities and differences—Both male IBS and male HCs showed strong positive connectivity between the aMCC and the aINS, compared to weak connectivity between these regions in females, possibly related to the greater engagement of the INS seen in male subjects seen in this and previous studies [6,52]. Both the aMCC and the aINS are also regions of a salience network, supporting the greater relevance of the fear and anger stimuli for males [62]. Male compared to female IBS subjects showed significant differences in the connectivity within the emotional arousal circuit, including stronger connectivity between the pACC/vmPFC to the AMYG and modulatory input of PFC subregions to pACC/vMPFC. While male IBS showed weak positive connectivity from the vlPFC, female IBS showed moderately strong input from dlPFC. Overall, greater engagement of emotional arousal circuit was observed in males.

4.2.2 Disease related similarities and differences—Both male and female IBS differed from their respective control groups in the connectivity between sgACC to pACC/vmPFC, and in the connectivity between aMCC and PCC, suggesting these differences to be IBS related. Male IBS subjects differed from male HCs in the connectivity between pACC/vmPFC to aMCC, which was stronger in IBS. Together with the stronger activation of the involved cingulate subregions, and the positive connectivity between aMCC and aINS, the

pACC/vmPFC to aMCC to aINS pathway may underlie the greater engagement of the INS in male subjects, previously reported in IBS [7,52]. Greater positive connectivity from HIPP to AMYG was also observed in male IBS versus male HCs, consistent with the greater engagement of the AMYG in male IBS. Female IBS differed from female controls in the connectivity between dlPFC and pACC/vmPFC, which was negative in HCs and positive in IBS.

4.3.0 Limitations

There are several limitations to the study: 1) Although we were able to show clear disease and sex related differences in the AMYG to a non-IBS related stimulus, there are some limitations to the emotional recognition task used. The faces task has been shown to elicit an immediate response in brain circuits involved in attention, cognition, and emotion [1,35], highlighting the highly adaptive social trait involved when recognizing and responding to emotional states of another individual [9]. While initially proposed to quantify individual differences in emotional reactivity [20], the task is not associated with subjective emotional feelings or autonomic nervous system responses, and is better viewed as an emotion recognition task which engages both cognitive and affective brain circuits [15,23,41,42]. 2) We were unable to compare the specific brain responses associated with fear and anger respectively, which could have highlighted sex-specific responses associated with differing negative emotions. 3) We were unable to assess possible effects of female sex hormones on brain responses in our sample of predominantly premenopausal females, scanned during the follicular phase of the menstrual cycle.

4.4.0 Summary and conclusions

When viewed together with previously published studies in female IBS subjects using GI specific stimuli [69], the current findings confirm the general concept that IBS subjects show sex-specific increased responsiveness to emotionally valenced stimuli, which are not fully accounted for by anxiety. This increased brain responsiveness to emotionally-valenced stimuli, may play a role in the process of central sensory amplification of visceral and non-visceral stimuli, characteristic for this patient population [70]. The fact that in males altered brain responsiveness could be elicited by a stimulus without any relevance to IBS or pain symptoms, is consistent with the concept that brain alterations play a role in IBS pathophysiology, and that IBS is not a gut specific disorder. Increased perception of visceral stimuli appears to be just one of several manifestations of this brain hyper-responsiveness. The findings emphasize the importance of taking sex-related differences into account, when evaluating disease differences and when assessing the importance of symptom related stressors in the clinic. While aversive pelvic stimuli or their expectation may be more salient to women (the majority of whom have experienced physiological pain in this area related to menstrual cycle and delivery), negative personal interactions involving recognition of potential adversaries may be more salient in males to elicit greater responses in cognitive affective brain circuits, and contribute to symptom variations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

IBS	Irritable bowel syndrome
HC	Healthy Control
M	male
F	female
ME	match emotions
MF	match forms
ROI	region-of-interest
AMYG	amygdala
ACC	anterior cingulate cortex
aMCC	anterior mid cingulate cortex
sgACC	subgenual cingulate cortex
pACC	pregenual cingulate cortex
PCC	posterior cingulate cortex
HIPP	hippocampus
HYPO	hypothalamus
PAG	dorsal pons
NACC	nucleus accumbens
PFC	prefrontal cortex
vlPFC	ventrolateral prefrontal cortex
vmPFC	ventromedial prefrontal cortex
dmPFC	dorsomedial prefrontal cortex
dlPFC	dorsolateral prefrontal cortex
INS	insula
aINS	anterior insula
mINS	mid insula
pINS	posterior insula
BSQ	UCLA Bowel Symptom Questionnaire
HAD	Hospital Anxiety and Depression Index

SPM	Statistical Parametric Mapping software
MNI	Montreal Neurological Institute

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Summary

Sex differences in the engagement of brain networks in response to negative emotional faces was investigated. Male IBS demonstrated differential engagement of emotional arousal circuits.

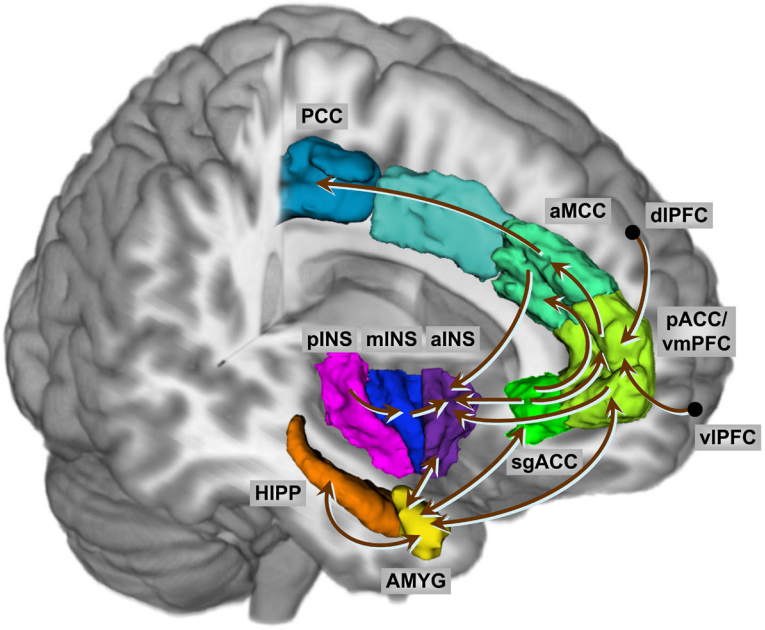

Figure 1. Cortical-Affective Circuit Effective Connectivity Model

Regions: AMYG, amygdala; aINS, anterior insula; mINS, mid insula; pINS, posterior insula; HIPP, hippocampus, aMCC, anterior mid cingulate cortex; sgACC, subgenual cingulate cortex; PCC, posterior cingulate cortex; pACC, pregenual cingulate cortex; vmPFC, ventro-medial prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; vlPFC, ventro-lateral prefrontal cortex

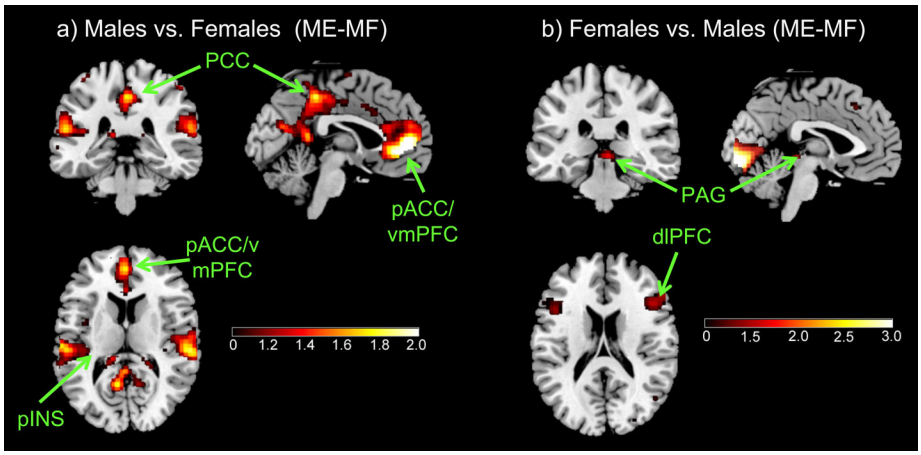

Figure 2. Sex Differences (IBS+HC) for (ME-MF)

a) Males vs. Females (ME-MF)

b) Females vs. Males (ME-MF)

IBS: Irritable bowel syndrome, HC: Healthy control

Regions: AMYG, amygdala; aINS, anterior insula; mINS, mid insula; pINS, posterior insula; HIPP, hippocampus, aMCC, anterior mid cingulate cortex; sgACC, subgenual cingulate cortex; PCC, posterior cingulate cortex; pACC, pregenual cingulate cortex; mPFC, medial prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; PAG, dorsal pons; NACC, nucleus accumbens.

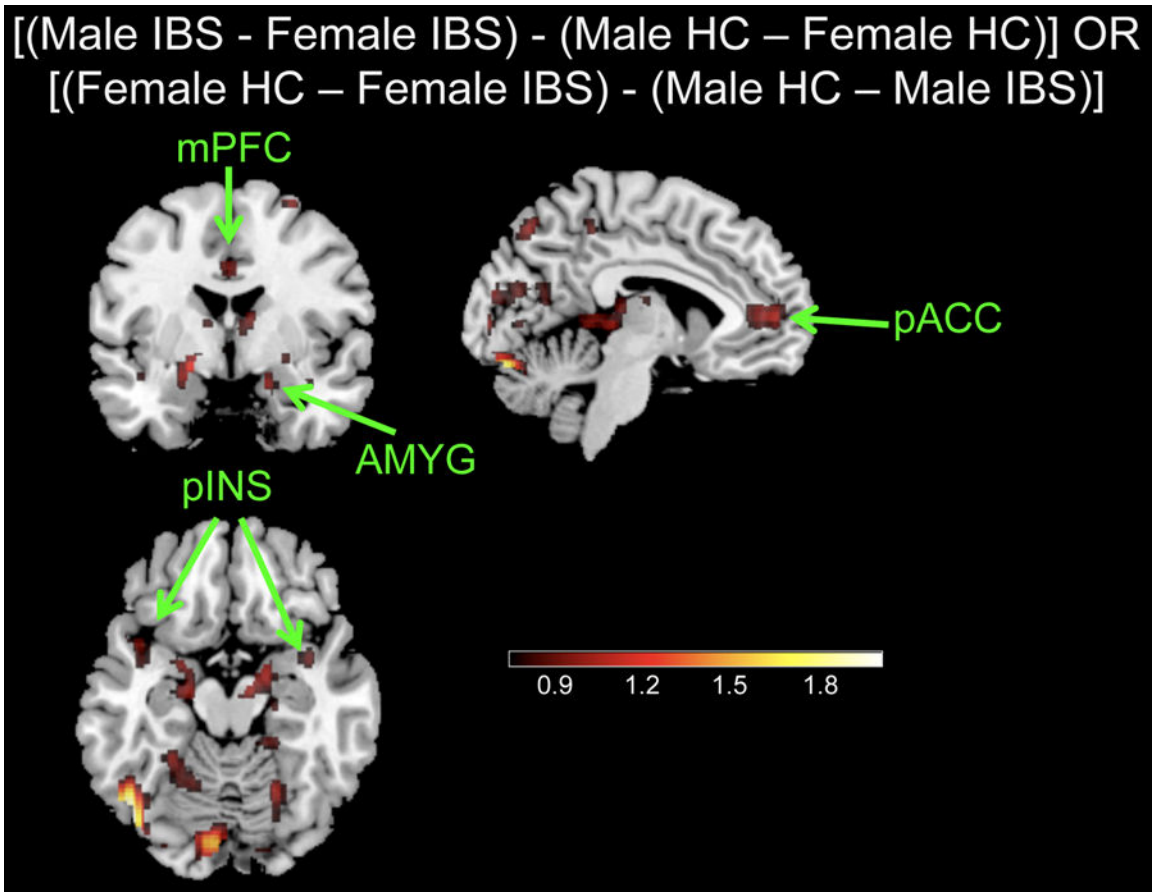

Figure 3. Interaction between sex and disease for (ME-MF)

[(Male IBS-Female IBS) – (Male HC-Female HC)] or [(Female HC-Female IBS) – (Male HC-Male IBS)]

IBS: Irritable bowel syndrome, HC: Healthy control

Regions: AMYG, amygdala; mINS, mid insula; pINS, posterior insula; HIPP, hippocampus; HYPO, hypothalamus; aMCC, anterior mid cingulate cortex; sgACC, subgenual cingulate cortex; PCC, posterior cingulate cortex; pACC, pregenual cingulate cortex; vmPFC, ventro-medial prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; NACC, nucleus accumbens.

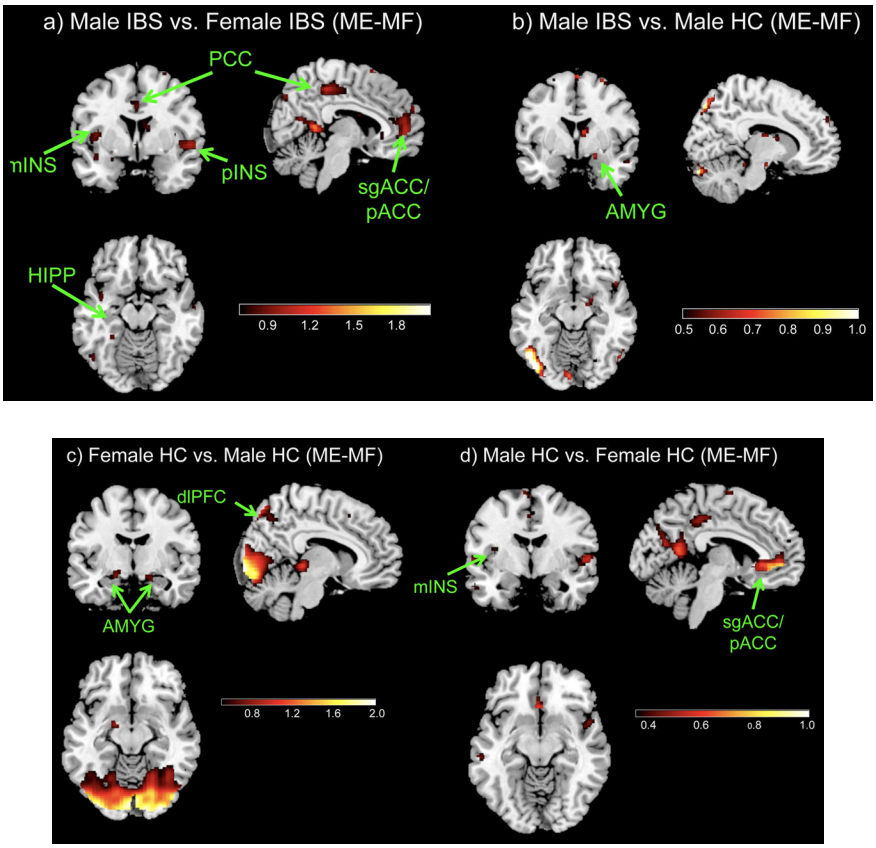

Figure 4. Disease and Sex Differences for (ME-MF)

a) Male IBS vs. Female IBS

b) Male IBS vs. Male HC

c) Female HC vs. Male HC

d) Male HC vs. Female HC

IBS: Irritable bowel syndrome, HC: Healthy control

Regions: AMYG, amygdala; mINS, mid insula; pINS, posterior insula; HIPP, hippocampus; HYPO, hypothalamus; aMCC, anterior mid cingulate cortex; sgACC, subgenual cingulate cortex; PCC, posterior cingulate cortex; pACC, pregenual cingulate cortex; vmPFC, ventro-medial prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; NACC, nucleus accumbens.

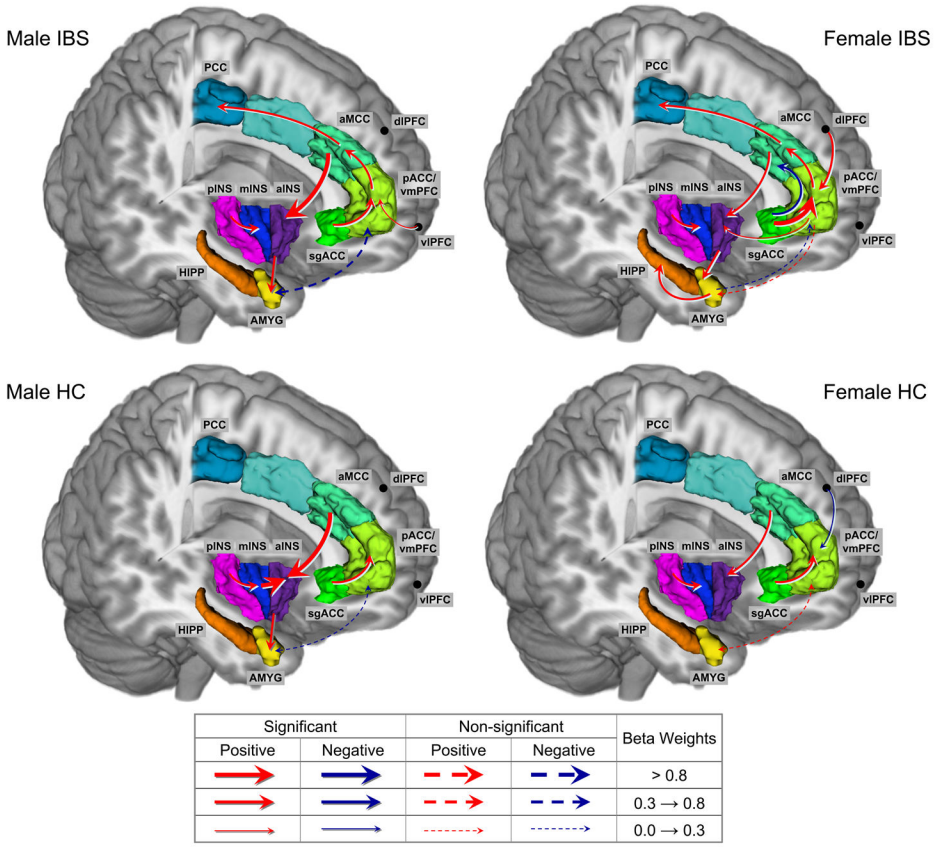

Figure 5. Effective Connectivity for (ME-MF) by Group (IBS Males, IBS Females, HC Males, HC Females)

IBS: Irritable bowel syndrome, HC: Healthy control

Regions: AMYG, amygdala; aINS, anterior insula; mINS, mid insula; pINS, posterior insula; HIPP, hippocampus, aMCC, anterior mid cingulate cortex; sgACC, subgenual cingulate cortex; PCC, posterior cingulate cortex; pACC, pregenual cingulate cortex; vmPFC, ventro-medial prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; dlPFC, dorsalateral prefrontal cortex

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Table 1

Study Demographics and Characteristics

	IBS		CONTROL			
	MALES	**FEMALES**	**MALES**	**FEMALES**	**F**	**p**
N	20	27	29	38	-	-
PREMENOPAUSAL	-	25	-	35	-	-
AGE (yrs)	36.05 (12.56)	31.41 (10.35)	35.61 (12.46)	31.74 (13.19)	a) 0.00 b) 3.39	a) .100 b) .068
IBS Symptom Severity	3.05 (0.76)	3.00 (0.62)	1.03 (0.19)	1.03 (0.16)	a) 27.35 b) 0.44	a) <.001** b) .508
ANXIETY (HAD)	6.80 (4.51)	5.59 (3.54)	2.83 (2.62)	3.63 (2.50)	a) 14.58 b) 4.26	a) <.001** b) .041*
DEPRESSION (HAD)	3.70 (3.7)	3.23 (2.55)	1.90 (2.09)	0.95 (1.11)	a) 21.43 b) 6.62	a) <.001** b) .011*

Measures: IBS Symptom Severity: 1=None; 2=Mild; 3=Moderate; 4=Severe; 5=Very Severe

HAD: Hospital Anxiety and Depression Measure

Premenopausal status was determined using a question and female subjects were classified as premenopausal and postmenopausal

Groups: IBS, Irritable Bowel Syndrome; HC, Healthy Controls

a) IBS vs. HC; b) Males vs. Females

Significance levels:

* p<.05,

** p<.001

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Table 2

Peak MNI Coordinates of Significant Activation for Males vs. Females for the Matched Emotions versus Matched Forms Contrast (ME-MF)

	Region	Hemisphere	Cluster Size	p value	q valu(FDR)e	Z	X mm	Y mm	Z mm
ME-MF									
Males-Females	mINS	Left	180	.007	.010	5.503	−38	0	10
	mINS	Right	281	.002	.003	4.826	36	4	6
	pINS	Left	528	.001	.001	4.925	−34	−26	14
	pINS	Right	432	.001	.002	4.866	36	−24	4
	mPFC	Left	860	.001	.001	5.618	−6	44	−2
	mPFC	Right	215	.012	.017	4.327	2	48	4
	dlPFC	Left	312	.023	.028	5.521	−40	2	10
	aMCC	Left	112	.026	.031	4.216	0	28	18
	aMCC	Right	127	.022	.028	4.362	2	30	18
	pACC	Left	995	.001	.001	5.618	−6	44	−2
	pACC	Right	589	.001	.001	4.823	0	34	−4
	sgACC	Left	116	.012	.015	5.492	−4	34	−4
	sgACC	Right	134	.010	.014	4.823	0	34	−4
	HIPP	Left	18	.049	.050	3.333	−28	−28	−14
	NACC	Left	3	.036	.040	4.748	−8	16	−4
	NACC	Right	7	.029	.033	3.742	10	12	−4
	PCC	Left	256	.001	.001	4.260	−2	−36	38
	PCC	Right	256	.001	.002	4.520	2	−24	38
Females-Males	dlPFC	Right	749	.001	.001	4.48	46	20	22
	PAG	Left	5	.022	.022	3.48	0	−28	−4
	PAG	Right	7	.027	.027	4.27	4	−30	−6

Regions: AMYG, amygdala; aINS, anterior insula; mINS, mid insula; pINS, posterior insula; HIPP, hippocampus, HYPO, hypothalamus, aMCC, anterior mid cingulate cortex; sgACC, subgenual cingulate cortex; pACC, pregenual cingulate cortex; PCC, posterior cingulate cortex; mPFC, medial prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; NACC, nucleus accumbens;

Groups: IBS, Irritable bowel syndrome; HC, Healthy Control

Contrasts: ME-MF, matching emotions versus matching forms

Coordinates are represented in MNI (Montreal Neurologic Institute) space with x, y, z coordinates

FDR: False discovery rate (correction for multiple comparisons)

Table 3

Peak MNI Coordinates of Significant Activation for the Interaction between Sex and Disease [(Male IBS-Female IBS) – (Male HC-Female HC)] Effects for the Matched Emotions versus Matched Forms Contrast (ME-MF)

	Region	Hemisphere	Set p value	q value (FDR)	Number of Set Clusters	Cluster Size	Z	X mm	Y mm	Z mm
ME-MF										
[(Male IBS – Female IBS) – (Male HC – Female HC)]										
	pINS	Left	<.001	.001	4	2	2.773	−36	−4	−18
			<.001	.001		2	2.763	−34	−10	−4
			<.001	.001		1	2.721	−36	−16	−10
			<.001	.001		1	2.692	−34	−12	−8
	pINS	Right	.039	.045	2	7	3.099	38	−2	−16
			.039	.045		19	2.962	36	−12	−2
	mPFC	Right	.024	.030	3	38	3.095	12	50	16
			.024	.030		22	2.901	20	60	28
			. 024	.030		5	2.754	2	40	12
	pACC	Left	.001	.002	4	27	2.812	0	40	10
			.001	.002		1	2.768	−18	48	6
			.001	.002		3	2.746	−2	30	8
			.001	.002		1	2.634	−8	34	6
	pACC	Right	.013	.017	3	35	3.096	12	50	16
			.013	.017		28	2.812	0	40	10
			.013	.017		1	2.697	0	30	8
	AMYG	Right	.005	.007	2	22	3.159	32	−2	−18
			.005	.007		11	3.063	18	−6	−18

Regions: AMYG, amygdala; aINS, anterior insula; mINS, mid insula; pINS, posterior insula; HIPP, hippocampus, HYPO, hypothalamus, aMCC, anterior mid cingulate cortex; sgACC, subgenual cingulate cortex; pACC, pregenual cingulate cortex; PCC, posterior cingulate cortex; mPFC, medial prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; NACC, nucleus accumbens;

Groups: IBS, Irritable bowel syndrome; HC, Healthy Control

Contrasts: ME-MF, matching emotions versus matching forms

Coordinates are represented in MNI (Montreal Neurologic Institute) space with x, y, z coordinates

FDR: False discovery rate (correction for multiple comparisons)

Table 4

Peak MNI Coordinates of Significant Activation for the Sex and Disease Effects for the Matched Emotions versus Matched Forms Contrast (ME-MF)

	Region	Hemisphere	Cluster Size	p value	q value (FDR)	Z	X mm	Y mm	Z mm
ME-MF									
Male IBS-Female IBS									
	mINS	Left	149	.001	.015	4.663	−42	0	8
	mINS	Right	263	.001	.005	4.099	40	−4	14
	pINS	Left	286	.001	.004	4.128	−36	−22	16
	pINS	Right	414	.001	.002	4.333	38	−24	6
	mPFC	Left	736	.001	.001	4.715	−6	46	2
	mPFC	Right	347	.005	.005	3.775	2	48	8
	dlPFC	Left	330	.019	.026	5.092	−40	2	10
	aMCC	Left	95	.033	.041	3.922	0	26	18
	aMCC	Right	131	.021	.026	3.993	2	30	16
	pACC	Left	972	.001	.001	4.715	−6	46	2
	pACC	Right	654	.001	.001	4.043	0	46	8
	sgACC	Left	107	.012	.018	3.876	−4	32	−2
	sgACC	Right	104	.015	.021	4.160	6	24	−2
	HIPP	Left	31	.001	.018	3.340	−28	−28	−14
	HYPO	Left	1	.001	.048	2.939	−10	−2	−2
	NACC	Left	3	.036	.042	3.869	−8	16	−4
	NACC	Right	1	.038	.042	2.812	8	18	−4
	PCC	Left	863	.001	.002	3.864	−12	−18	46
	PCC	Right	863	.001	.002	4.115	8	−30	48
Male HC–Female HC									
	mINS	Left	44	.047	.050	2.772	−34	−4	14
	pACC	Left	236	.008	.014	4.113	−4	34	−4
	pACC	Right	132	.027	.041	3.5557	0	32	−6
	sgACC	Left	101	.013	.022	4.113	−4	34	−4
	sgACC	Right	84	.020	.031	3.628	0	26	−8
	NACC	Left	2	.038	.049	2.772	−10	18	−4
	NACC	Right	5	.031	.046	3.157	10	12	−4

	Region	Hemisphere	Cluster Size	p value	q value (FDR)	Z	X mm	Y mm	Z mm
Female HC–Male HC									
	dlPFC	Left	1059	.001	.001	5.648	−44	14	24
	PAG	Left	6	.021	.026	3.707	0	−28	−4
	PAG	Right	7	.027	.032	4.157	4	−30	−6
Male IBS–Male HC									
	AMYG	Right	11	.045	.050	2.830	30	0	−22
	AMYG	Right	22	.047	.079	3.159	32	−2	−18

Regions: AMYG, amygdala; aINS, anterior insula; mINS, mid insula; pINS, posterior insula; HIPP, hippocampus, HYPO, hypothalamus, aMCC, anterior mid cingulate cortex; sgACC, subgenual cingulate cortex; pACC, pregenual cingulate cortex; PCC, posterior cingulate cortex; mPFC, medial prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; NACC, nucleus accumbens;

Groups: IBS, Irritable bowel syndrome; HC, Healthy Control

Contrasts: ME-MF, matching emotions versus matching forms

Coordinates are represented in MNI (Montreal Neurologic Institute) space with x, y, z coordinates

FDR: False discovery rate (correction for multiple comparisons)

Table 5a

Beta weights and Confidence Intervals for Disease and Sex Effects for the Matched Emotions versus the Matched Forms Contrast (ME-MF).

Matching Emotions –Matching Forms (ME-MF)								
	Male HC		Female HC		Male IBS		Female IBS	
	Beta	CI (L-U)	Beta	CI (L-U)	Beta	CI (L-U)	Beta	CI (L-U)
A. Affective Limbic Circuit								
sgACC → AMYG	−0.13	−0.67 0.25	−0.18	−0.58 0.14	0.25	−0.51 0.77	−0.24	−0.86 1.01
AMYG → sgACC	0.13	−0.14 0.42	−0.19	−0.84 0.14	0.24	−0.26 1.00	−0.23	−0.70 0.51
sgACC → pACC/vmPFC	0.51	0.08 1.00	0.54	0.26 1.03	0.48	0.11 1.21	1.39	0.53 2.17
pACC/vmPFC → AMYG	−0.20	−0.61 0.21	0.11	−0.50 0.52	−0.39	−1.38 0.49	0.24	−0.75 0.71
pACC/vmPFC → aINS	0.16	−0.33 0.57	−0.13	−0.41 0.37	0.58	−0.59 1.39	0.25	−0.09 0.71
sgACC → aMCC	0.16	−0.14 0.43	0.01	0.23 0.23	−0.13	0.58 0.92	−0.44	−1.04 0.00
aMCC → aINS	0.82	0.25 1.71	0.42	0.01 0.74	0.99	… 1.45	0.41	0.07 0.81
AMYG → aINS	−0.20	−0.76 0.43	0.01	−0.36 0.42	0.09	−0.48 0.97	0.02	−0.55 0.53
aINS → AMYG	0.52	−0.01 0.83	0.40	−0.22 0.91	0.39	… 0.58	0.78	−0.35 1.37
B. Prefrontal and Hippocampal Modulatory Input to Affective Limbic Circuit								

Matching Emotions –Matching Forms (ME-MF)								
	Male HC		Female HC		Male IBS		Female IBS	
	Beta	CI (L-U)	Beta	CI (L-U)	Beta	CI (L-U)	Beta	CI (L-U)
dlPFC →pACC/vmPFC (to amyg)	−0.12	−0.59 0.18	−0.22	−0.62 0.15	0.03	−0.28 0.73	0.78	−0.59 1.89
vlPFC → pACC/vmPFC (to amyg)	0.06	−0.62 1.22	−0.05	−0.74 0.39	0.24	… 0.59	−0.01	−0.69 0.77
HIPP → AMYG	0.78	−0.09 1.54	0.32	−0.25 1.05	−0.03	−0.84 1.00	−0.14	−1.06 2.18
AMYG → HIPP	0.16	−0.01 0.39	0.04	−0.29 0.25	0.16	−0.18 0.54	0.64	−0.79 1.49
C. Intrainsular Connectivity								
pINS → mINS	0.41	0.24 0.56	0.57	0.22 1.09	0.60	0.20 1.14	0.57	0.15 1.08
mINS → aINS	0.89	−0.01 1.95	−0.04	−0.58 0.33	0.29	−0.86 1.48	0.12	−0.53 0.79
sgACC → aINS	−0.28	−0.59 0.11	−0.04	−0.41 0.22	−0.43	−1.47 0.59	−0.27	−0.69 0.22
D. Intracingulate Connectivity								
AMYG → pACC/vmPFC	−0.01	−0.45 0.53	0.07	−0.30 0.57	−0.46	−0.87 …	−0.24	−0.94 2.25
pACC/vmPFC → aMCC	−0.19	−0.45 0.13	0.00	−0.42 0.35	0.69	−0.27 1.66	0.38	−0.30 0.63
aMCC → PCC	0.00	−0.46 0.82	0.21	−0.27 0.83	0.51	−0.29 0.79	0.72	0.29 1.12

Critical Values: Uncorrected: χ^2=6.63, p < .01, χ^2=3.84, p < .05; significance after FDR corrected:

** p < .01 corrected,

* p < .05 corrected (Male IBS vs. Female IBS, Male HC vs. Female HC, df=1)

Bootstrapped CI(L-U): confidence interval- lower to upper limit, 95% bias corrected

Beta weights: weak (β=0 to.30), moderate (β=30 to.80), or strong (β= >.80).

… When AMOS was unable to estimate the upper or lower bound confidence limits

Regions: AMYG, amygdala; aINS, mINS, mid insula; pINS, posterior insula; HIPP, hippocampus, aMCC, anterior mid cingulate cortex; sgACC, subgenual cingulate cortex; PCC, posterior cingulate cortex; pACC, pregenual cingulate cortex; vmPFC, ventro-medial prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; vlPFC, ventro-lateral prefrontal cortex

Groups: IBS, Irritable bowel syndrome; HC, Healthy Control

Contrasts: ME, matching emotions; MF, matching forms

Table 5b

χ^2 differences Disease and Sex Effects for the Matched Emotions versus the Matched Forms Contrast (ME-MF).

Matching Emotions-Matching Forms (ME-MF)			
	Male IBS vs. Female IBS X^2 difference	**Male IBS vs. Male HC X^2 difference**	**Female IBS vs. Female HC X^2 difference**
A. Affective Limbic Circuit			
sgACC → AMYG	**3.9**	2.5	0.0
AMYG → sgACC	3.2	0.2	0.0
sgACC → pACC/vmPFC	**13.5****	0.0	**11.7****
pACC/vmPFC → AMYG	**5.6**	0.5	0.4
pACC/vmPFC → aINS	0.6	0.9	**7.6****
sgACC → aMCC	1.9	2.3	**7.2****
aMCC → aINS	**4.4**	0.3	0.0
AMYG → aINS	0.1	0.9	0.0
aINS → AMYG	2.6	0.5	2.2
B. Prefrontal and Hippocampal Modulatory Input to Affective Limbic Circuit			
dlPFC → pACC/vmPFC (to amyg)	**5.9**	1.0	**8.2****
vlPFC → pACC/vmPFC (to amyg)	0.9	0.5	0.0
HIPP → AMYG	0.2	**4.5**	**3.8**
AMYG → HIPP	**9.3****	0.0	**13.0****
C. Intrainsular Connectivity			
pINS → mINS	0.0	1.0	0.0
mINS → aINS	0.2	1.5	0.6
sgACC → aINS	0.3	0.0	0.0
D. Intracingulate Connectivity			
AMYG → pACC/vmPFC	0.4	2.4	1.0
pACC/vmPFC → aMCC	1.3	**10.8****	**9.3****
aMCC → PCC	1.3	**6.5**	**6.9****

Significance: Uncorrected: χ^2=6.63, p < .01, χ^2=3.84, p < .05; significance after FDR corrected:

** p < .01 corrected,

* p < .05 corrected (Male IBS vs. Female IBS, Male HC vs. Female HC, df=1)

FDR: False discovery rate (controlling false discovery rate at 5%)

Regions: AMYG, amygdala; aINS, mINS, mid insula; pINS, posterior insula; HIPP, hippocampus, aMCC, anterior mid cingulate cortex; sgACC, subgenual cingulate cortex; PCC, posterior cingulate cortex; pACC, pregenual cingulate cortex; vmPFC, ventro-medial prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; vlPFC, ventro-lateral prefrontal cortex

Groups: IBS, Irritable bowel syndrome; HC, Healthy Control

Contrasts: ME, matching emotions; MF, matching forms