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## Irritable Bowel Syndrome in female patients is associated with alterations in structural brain networks

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### Abstract

Alterations in gray matter (GM) density/ volume and cortical thickness (CT) have been demonstrated in small and heterogeneous samples of subjects with different chronic pain syndromes, including irritable bowel syndrome (IBS). Aggregating across 7 structural neuroimaging studies conducted at UCLA between August 2006 and April 2011, we examined group differences in regional GM volume in 201 predominantly premenopausal female subjects (82 IBS, mean age:  $32 \pm 10$  SD, 119 Healthy Controls [HCs],  $30 \pm 10$  SD). Applying graph theoretical methods and controlling for total brain volume, global and regional properties of large-scale structural brain networks were compared between IBS and HC groups. Relative to HCs, the IBS group had lower volumes in bilateral superior frontal gyrus, bilateral insula, bilateral amygdala, bilateral hippocampus, bilateral middle orbital frontal gyrus, left cingulate, left gyrus rectus, brainstem, and left putamen. Higher volume was found for the left postcentral gyrus. Group differences were no longer significant for most regions when controlling for Early Trauma

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#### Authors' contributions

All authors made substantive intellectual contributions to this research and development effort including the article conception, study design, data processing, drafting and revising the final manuscript.

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As of September 2013, the Laboratory of Neuro Imaging (LONI) will be relocated to the University of Southern California (USC). Thus, some of the URL links, web-page references, and internet resources cited throughout this manuscript may be relocated to appropriate subdomains under <http://www.loni.usc.edu>. If you find broken links or defunct URLs please contact [help@loni.usc.edu](mailto:help@loni.usc.edu).

Inventory global score with the exception of the right amygdala and the left post central gyrus. No group differences were found for measures of global and local network organization. Compared to HCs, the right cingulate gyrus and right thalamus were identified as significantly more critical for information flow. Regions involved in endogenous pain modulation and central sensory amplification were identified as network hubs in IBS. Overall, evidence for central alterations in IBS was found in the form of regional GM volume differences and altered global and regional properties of brain volumetric networks.

## Keywords

chronic pain; irritable bowel syndrome; gray matter volume; brain network analysis; graph theory

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### 1.1.0. Introduction

Structural alterations of the brain in the form of higher or lower gray matter (GM) density and volume and cortical thickness (CT) have been reported in a wide range of chronic somatic and visceral pain conditions, including chronic inflammatory conditions (osteoarthritis [6; 107], chronic pancreatitis [49; 128], Crohn's disease [2]), persistent often comorbid pain syndromes (temporomandibular disorder [51; 54; 95; 115; 144], vulvodinia [115], chronic pelvic pain [5; 97], irritable bowel syndrome [12; 35; 116], fibromyalgia [20; 75; 143]), migraine [71; 106; 111; 141], chronic tension-type headache [113], cyclical menstrual pain [138]) and other pain conditions (chronic lower back pain [4; 99; 112; 139], complex regional pain syndrome [50; 53; 54; 99].) The variability in reported findings may be in part related to the fact that the majority of these studies were relatively small, and poorly controlled for medication intake, the presence of comorbid conditions, or sex. The most commonly reported regions with lower GM were subregions of cingulate and insular cortices, temporal lobe, prefrontal cortex and thalamus/basal ganglia [34; 88], while disease related regional GM volume increases have been reported in a few studies [94; 115; 116], involving basal ganglia, hippocampus, anterior cingulate (ACC) subregions, posterior insula, and somatosensory cortex (S1). Previously published reports suggest different patterns of grey matter changes for different types of chronic pain, such as neuropathic and nociceptive pain [53; 54].

Recent research examining GM morphometry (volume, surface area, cortical thickness), anatomical connectivity (white matter tractography) and brain function (resting state, task-based) indicate that disease related symptoms may be associated with altered integration of brain regions that comprise large-scale networks [8; 9; 17; 19; 26; 57–59; 108; 110]. In studies examining GM morphometry, anatomical connectivity is inferred from correlated regional GM measures (e.g., volumes). Correlation of anatomic features of brain regions across individuals may partly reflect functional interactions between these areas [19; 121], mutually trophic effects on growth mediated by axonal connections [45–47], tissue type similarities [19; 27], genetics [24; 45; 114; 130], and/or environment-related plasticity [1; 45; 92; 114].

We aggregated data across 7 structural neuroimaging studies conducted at UCLA between August 2006 and April 2011, and examined group differences in regional GM volume in a

large sample of 201 female subjects (82 IBS, 119 HCs). We applied network analysis to obtain new insights about large-scale regional connectivity and to compare morphological brain architectures and network properties between groups of IBS and HC subjects. To assist in our large-scale analyses we employed the Laboratory of Neuro Imaging (LONI) pipeline [39; 41; 136], a graphical workflow environment which allows users to describe executable tools in a graphical user interface and create processing modules as nodes in a graph representing the complete computational protocol [40; 42]. We provide evidence for both regional alterations in GM volume, as well as differences in regional properties of large scale structural brain networks in IBS compared to HCs.

## 2.1.0. Methods

**2.1.1. Subjects**—Female IBS and HC subjects were recruited from multiple clinical sites at UCLA, which are part of the clinical research network of the Center for Neurobiology of Stress, and from community advertisements. A diagnosis of IBS was made using the ROME II or III symptom criteria [86; 131] based on assessments by gastroenterologists experienced in the diagnosis of functional bowel disease and the exclusion of organic disease. A gastroenterologist or GI nurse practitioner obtained a history and conducted a physical examination. IBS patients with all types of predominant bowel habits were included. Using clinical histories or questionnaire data, subjects with a history of any chronic functional symptom or syndrome, or symptoms suggestive of disordered mood or affect were excluded. Potential subjects were also excluded if they a) had a serious medical condition or were taking medications, as this may interfere with interpretation of the brain imaging or physiological measures; b) had an ongoing major psychiatric diagnosis or were being treated with psychotropic medication being used over the past 6 months (subjects were not excluded for lifetime incidence of psychiatric disorders, or for intake of low dose tricyclic antidepressants for non-psychiatric indication); c) engaged in excessive physical exercise (i.e. marathon runners).

**2.1.2. Structural MRI**—Brain images from 201 females (82 IBS, 119 HCs) were obtained and combined from 7 structural imaging studies conducted at UCLA using four separate protocols. Included in this subject pool, were 55 IBS and 48 HCs included in a prior analysis of GM density using voxel-based morphometry [116]. Brain images were acquired on 1.5 or 3T MRI scanners (Siemens Allegra). First, a sagittal scout was used to position the head. Then each subject underwent one of 4 structural acquisition sequences using a high-resolution 3-dimensional T1-weighted, sagittal, magnetization prepared rapid gradient echo (MP-RAGE) protocol. See Tables 1 and 2 for a description of the structural acquisition protocols.

**2.1.3. Phenotype data**—We collected phenotyping data on early adverse life events (EALs) (early trauma inventory; ETI) [15], trait anxiety scores (State Trait Anxiety Inventory; STAI) [122], depression and anxiety (Hospital Anxiety and Depression Scale; HAD) [145], catastrophizing (Coping Strategies Questionnaire; CSQ) [105], and health status (Patient Health Questionnaire; PHQ, using 12 items without GI related questions) [73]. IBS symptom severity, abdominal pain and duration in the past week were measured

on a 21-point numeric rating scale (Bowel Symptoms Questionnaire; BSQ) [98]. Usual symptom severity was assessed on an ordinal scale (1=None; 2=Mild; 3=Moderate; 4=Severe; 5=Very Severe). Menopause status was assessed by self-report.

## 2.2.0. Data Analyses

**2.2.1. Volumetric Analysis**—The LONI (UCLA Laboratory of Neuroimaging) pipeline was used for image preprocessing and volumetric analysis. Figure 1 shows the pipeline workflow implementing the volumetric data analysis. The volumetric workflow pipeline consists of 4 main components— data preprocessing (intensity inhomogeneity correction [118] and skull-stripping [83; 84], cortical surface modeling [48], and tissue classification [134; 135]. The complete pipeline workflows are available as XML objects which can be downloaded, viewed and tested via the Pipeline environment ([pipeline.loni.ucla.edu](http://pipeline.loni.ucla.edu)), see supplementary Files 1 and 2.

**2.2.2. Brain Parcellation**—Global and regional volumetric analyses rely on dividing the brain into both its different tissues and 56 predefined brain structures based on the LONI probabilistic brain atlas (LBPA 40) [118] using SVPAsseg [134; 135]. In order to analyze additional subcortical brain regions not delineated by the above method, we applied FSL FMRIB's Integrated Registration and Segmentation Tool (FIRST v1.2) [66; 120], and the Harvard-Oxford atlas (amygdala, thalamus, nucleus accumbens) [37]. Table 3 contains the list of regions and an interactive LBP40 atlas parcellation depicting these regions can be examined at <http://scalablebrainatlas.incf.org/main>. For each tissue or brain structure the individual volume was measured by the voxel dimensions (1×1×1 mm) multiplied by the number of voxels within the segmented structure, revealing total brain volume (TBV), whole-brain grey matter (GM) volume or other regional volume. For each subject, regions of interest (ROIs) were visually inspected (using 3D viewer) after the brain parcellation. Then the across-subjects distributions volumetric measures defined on the 56 ROIs were quantitatively generated and inspected. Linear contrasts analyses on the estimates from a general linear model (GLM) were used to compare potential protocol differences in tissue classification controlling for group differences. The raw probability values of rejecting the null hypothesis due to chance alone (p-values) were adjusted for multiple comparisons, yielding a q-value, using a publicly available tool (<http://users.ox.ac.uk/~npike/fdr/>) to implement a graphically sharpened method for controlling false discovery rate at 5% (number of comparison=6) [103].

**2.2.3. Regions of Interest (ROI) for volumetric group comparisons**—To examine group differences and brain-symptom correlations, ROIs were selected based on functional neuroimaging studies in IBS, which have examined key regions of homeostatic afferent (interoceptive), emotional arousal and cognitive modulatory brain circuits [78; 79; 132; 133]. Briefly, the homeostatic afferent network is comprised of regions central for processing of viscerosensory information including thalamus, insula (posterior, mid, anterior), dorsal anterior cingulate [ACC] subregions [30; 31; 76; 79; 91]. The emotional arousal network includes amygdala, anterior insula, ACC subregions (including sub and pregenual), and dorsal pons (including the locus coeruleus complex) [62; 77; 102; 127; 132; 140]. The cognitive modulatory network reflects the modulating influence of cortical

regions (i.e. prefrontal, parietal) on the homeostatic afferent and emotional arousal networks [78; 90; 100]. From the LPBA 40 atlas [117], we examined superior, inferior and middle frontal gyrus, lateral and middle orbitofrontal gyrus (mOFG), cingulate, insula, brainstem, parahippocampal gyrus, gyrus rectus, hippocampus, putamen, and post central gyrus; and from the Harvard-Oxford atlas we examined amygdala, and thalamus.

Group differences in ROI volumes were determined using the general linear model (GLM) and linear contrast analysis controlling for TBV in SPSS v19. To determine if variations in group differences were explained by covariates trait anxiety (STAI) and early life trauma (ETI), we recalculated the original GLMs after adding each single covariate. Inspection of STAI and ETI indicated positive skewness in these measures. As such in order to improve the normality of the distribution, we transformed these scores using the square root transformation. We implemented the aforementioned graphically sharpened method for controlling false discovery rate at 5% (number of comparison=29) [103]. We quantify group differences by calculating Cohen's *d* reflecting the difference between HCs and IBS scale of standard deviation units. As a rule of thumb, an effect size of  $g=.80$  is considered large (explaining 14% of the variance),  $.50$  medium (6% variance explained), and  $.20$  small (1% variance explained).

In IBS, exploratory partial correlation analyses were conducted, controlling for total GM volume and age, in order to examine the relationship between regional GM volume and the continuous symptom measures data assessed by the BSQ, overall symptom severity and abdominal pain in the past week and chronicity of IBS symptoms.

**2.2.4 Complex Network Analysis**—For a detailed description of brain network measures see [108]. To investigate alterations in the architecture of structural networks in IBS compared to HCs we applied graph theoretical methods using the Graph Analysis Toolbox [60], which integrates the Brain Connectivity toolbox [108] for computing network measures. The analytical pipeline is depicted in Figure 2. Controlling for TBV with the linear regression, inter-regional volume based correlation matrices were computed for the 62 regional volumes for each group based on the parcellations using the LPBA 40 and Harvard-Oxford atlases. Volumetric-based brain networks were then constructed by binarizing the correlation matrices based on the minimum network density threshold in each group. The minimum density threshold is the correlation value at which all the regions or nodes of the network are fully connected to the brain networks in each group (i.e., none of the networks are fragmented). Choosing to threshold at the minimum density ensures results are not influenced by group differences in average degree and connection density. *Density* is the ratio of the number of connections present to the number of possible connections and is a measure of *wiring cost*. The connectivity or wiring of regions is thought to be based on the principle of wiring minimization and cost efficiency which permits the most economical use of space and energy (metabolic costs) in the brain [18]. *Minimum density* reflects the minimum *wiring cost* possible before network fragmentation (See INSET Figure 2). Consistent with previous studies [60; 61; 85], we chose the *minimum density* value to threshold the networks. However, because choice of threshold can affect measures of network topology [142], we applied a sensitivity analysis to examine global network properties at a range of *wiring costs* or *densities*,  $.05$  to  $.45$ . The binarized matrices entered

as data in the analysis are called *adjacency* or connection matrices and an entry of 1 reflects presence of a connection ( $r \geq$  minimum density) and 0 reflects absence of a connection ( $r <$  minimum density).

After generating the structural brain networks, network measures that characterize global and local brain efficiency were computed. Specifically, we examined the *small-world attributes* of the structural networks by examining the *clustering coefficient*, *characteristic path length*, and the *small-worldness* index. The *clustering coefficient* of a region quantifies the probability that the regions a particular brain region is connected to are also connected to each other. Averaging the *cluster coefficient* across all regions produces the *network's clustering coefficient*, which is considered an indicator of network segregation.

*Characteristic path length* refers to the average number of distinct *paths* (also called edges) for a signal to travel between two regions. A short *path length* indicates that a brain region can be reached from another region through a *path* composed of only a few connections. High clustering (i.e., complexity) and short *path lengths* (i.e., wiring) are properties of small-world attributes [123; 124]. We assessed *modularity* of the network, where higher *modularity* reflects several densely connected nodes and fewer connections between nodes in a different module [93]. In addition, *global efficiency* was quantified by using the inverse of the distance matrix, which represents the shortest possible *path* from each region to every other region [80].

Regional network properties were also calculated including *centrality*, *degree*, and *betweenness centrality*. *Centrality* represents the relative importance of a brain region within the network. *Degree* is the simplest measure of *centrality*. *Degree* is the number of regions connected to a region and regions with high *degree* are considered essential for maintaining global connectedness. *Betweenness centrality* refers to the fraction of shortest *paths* that pass through a region and regions with high *betweenness centrality* are considered critical for information flow. The *clustering coefficient* for each node was also computed, reflecting the fraction of a region's neighbor's that are also neighbors with each other and are thought to be key nodes for clusters or modules in the brain. Finally, *network hubs* were identified as regions whose *regional betweenness/degree* was 2 standard deviations greater than the average regional *betweenness/degree*. Hubs are considered important controllers of information flow and critical for efficient network communication.

Network parameters were quantified with respect to null-hypothesis networks having a random topology but sharing the same number of regions, connections, and *degree* (number of connections) distribution. To test for between-group differences in global and regional network measures, a non-parametric permutation test with 1000 repetitions was performed resulting in a permuted distribution of differences [60]. The p value for the group differences in regional measures was then calculated based on the percentile position of the group difference on the permuted distribution. We did not implement error control for multiple regions tested. Regional network measures were only compared at the minimum network density.

**2.2.5. Data visualization**—Pajek software (version 2.03) [36] was used to visualize the *betweenness* of structural brain network in IBS and HCs. A node represented each ROI, and



two regions with significant correlation (positive or negative) were linked by an edge. A Kamada–Kawai algorithm [67], a “spring embedding algorithm” was applied to create and optimize the structural network layout by minimizing variation in edge length [36].

### 3.1.0. Results

#### 3.1.1. Sample Characteristics

Clinical and behavioral variables for IBS and HCs are shown in Tables 4 and 5. Patients had significantly higher levels of state and trait anxiety, symptoms of depression, and catastrophizing than HCs. Even though elevated, average levels of anxiety and depression symptoms were within the normal range. The sample was predominantly premenopausal 145 (72%), ranging in age from 18 to 52. Within groups, 90% HCs and 93% IBS were premenopausal. Four participants (2%) reported being perimenopausal (HC=3(3%), IBS=1(2%)), ages ranging from 35 to 50, and 9 (4%) reported being postmenopausal (age range, 48 to 64, HCs=6 (7%), IBS=3 (4%)). Forty-two (21%) females, ages ranging from 18 to 56 did not respond to the question. Of these 42 subjects, only 1 IBS patient was older than 55 years.

#### 3.2.1. MRI Acquisition protocols and whole brain tissue classification

The 7 studies were comprised of 4 separate acquisition protocols, and the description of the acquisition protocols as well as the distribution of subjects and protocols across studies are described in Table 1 and 2. Linear contrasts analysis using GLM estimates comparing potential protocol differences in tissue classification across groups indicated no statistically significant differences in total GM or TBV between sites after adjusting p values to correct for number of contrasts performed. Linear contrasts indicated no protocol differences in total GM ( $q$ 's ranged from .11 to .94) or TBV (all  $q$ 's ranged from .71 to .93). We also found no indication of protocol\*group interaction effects. Figure 3 depicts TBV and TGM by protocol and group.

#### 3.2.2. Volumetric analysis comparing regions of interest between IBS and HCs

One subject failed in the LONI Pipeline workflow leaving 201 subjects available for the group analysis. Volumetric analysis demonstrated several highly significant moderate effect size *reductions* in regional GM volume in IBS patients compared to HCs, including bilateral superior frontal gyrus, bilateral insula, bilateral amygdala, bilateral hippocampus, bilateral middle orbital frontal gyrus, left cingulate, left gyrus rectus, brainstem, and left putamen (See Table 6, Figure 4). *Higher* GM volume was only seen in the left post-central gyrus (S1).

#### 3.2.3. Effects of trait anxiety and early life events (ETI global score) on volumetric changes

Group differences were reexamined after controlling for trait anxiety and ETI score (See Table 6). Neither anxiety nor ETI global scores were found to be significant predictors based on uncorrected p values. However, when controlling for ETI global score, group differences were no longer significant for most regions except for the right amygdala and the L post central gyrus. These findings suggest a relationship between GM volume and ETI score. Group differences were generally found to be robust to the inclusion of trait anxiety as a covariate.

### 3.2.4. Correlations of volumetric changes with GI symptoms in IBS

Exploratory correlation analysis controlling for TBV found small effect size associations between higher abdominal pain reports and overall symptom severity and lower volumes in left inferior frontal gyrus ( $r=-.26$ ,  $p=.02$ ;  $r=-.26$ ,  $p=.02$ ), left middle orbital frontal gyrus ( $r=-.28$ ,  $p=.01$ ;  $r=-.29$ ,  $p=.01$ ), and left lateral orbital frontal gyrus ( $r=-.31$ ,  $p=.006$ ;  $r=-.33$ ,  $p=.004$ ). Higher volume in the left superior frontal gyrus ( $r=-.26$ ,  $p=.02$ ) was associated with more abdominal pain in IBS. Greater duration of IBS was negatively associated with right inferior frontal gyri ( $r=-.26$ ,  $p=.02$ ). Lower volumes in left insula was also associated with greater overall symptom severity ( $r=-.26$ ,  $p=.03$ ).

#### 3.3.1. Network analysis

The *minimum density* threshold, ( $D=.17$ ) was achieved by thresholding the interregional volumetric correlation matrix at  $r=.32$  for IBS and  $r=.25$  for HC. The thresholded interregional covariance matrices used to create volumetric networks are depicted in Figure 5.

**Global organization**—Both HC and IBS structural networks were considered small world networks as the *small-worldness* index was greater than 1 across the entire range of *densities*. (See Figure 6), No group differences were found for other measures of global and local network organization (*characteristic path length*, *clustering*, *modularity*, *efficiency*) across the range of *wiring costs* or at the *minimum density* (see Table 7).

**Regional organization**—At the *minimum density*, we performed group comparisons of several measures indicative of how much impact a particular brain region exerts on the whole brain structural network including normalized *degree*, normalized *clustering coefficient*, and normalized *betweenness*. In terms of regions which were considered essential for maintaining global connectedness, right cingulate (*degree* ( $D$ ),  $D_{HC}=4$ ,  $D_{IBS}=16$ ,  $p=.044$ ) and the right lateral orbital frontal gyrus ( $D_{HC}=6$ ,  $D_{IBS}=13$ ,  $p=.045$ ) had significantly greater *degree* in IBS whereas the right angular gyrus ( $D_{HC}=12$ ,  $D_{IBS}=2$ ,  $p=.042$ ), and the left cingulate gyrus ( $D_{HC}=25$ ,  $D_{IBS}=21$ ,  $p=.048$ ) and the right fusiform gyrus ( $D_{HC}=23$ ,  $D_{IBS}=17$ ,  $p=.041$ ) had significantly greater *degree* in HCs compare to IBS.

Brain regions at the intersection of many *paths* and therefore capable of controlling information are characterized by high regional *betweenness*. IBS compared to HCs showed significantly higher *betweenness* for the right cingulate gyrus (*betweenness* ( $B$ ),  $B_{HC}=4.7$ ,  $B_{IBS}=96.7$ ,  $p=.029$ ), and right thalamus ( $B_{HC}=108.1$ ,  $B_{IBS}=356.4$ ,  $p=.020$ ). On the other hand, HCs showed significantly greater *betweenness* for the right inferior temporal gyrus ( $B_{HC}=236$ ,  $B_{IBS}=39.2$ ,  $p=.010$ ), left cingulate ( $B_{HC}=133.57$ ,  $B_{IBS}=33.40$ ,  $p=.036$ ), left ( $B_{HC}=389.8$ ,  $B_{IBS}=37.7$ ,  $p=.020$ ) and right hippocampus ( $B_{HC}=409.5$ ,  $B_{IBS}=122.1$ ,  $p=.040$ ).

In IBS compared to HC, the *clustering coefficient* for the left cingulate gyrus (*clustering coefficient* ( $C$ ),  $C_{HC}=.61$ ,  $C_{IBS}=.75$ ,  $p=.017$ ), right caudate ( $C_{HC}=.67$ ,  $C_{IBS}=.83$ ) and left hippocampus ( $C_{HC}=.50$ ,  $C_{IBS}=.74$ ,  $p=.029$ ) was greater, indicating these regions are most densely connected with other regions and likely key for brain modules or clusters in IBS. In



HCs, the right middle temporal gyrus ( $C_{HC}=1.0$ ,  $C_{IBS}=.33$ ,  $p=.039$ ) had a greater *clustering coefficient* compared to IBS.

Finally, *network hubs* were identified as regions whose *regional betweenness/degree* was 2 standard deviations greater than the average regional *betweenness/degree*. The average regional *degree* (SD) was  $D_{HC}=11(8.1)$  and  $D_{IBS}=11(7.4)$ . The average *betweenness* for was  $B_{HC}=97.16(104.2)$  and  $B_{IBS}=88.8(102.4)$ . Network hub analysis indicated that the right parahippocampal gyrus, ( $B_{HC}=289.6$ ,  $B_{IBS}=259.6$ ) and left lingual gyrus ( $B_{HC}=311.4$ ,  $B_{IBS}=460.0$ ) were important *hubs* in both groups. For IBS, additional *hubs* included brainstem ( $B=384.3$ ), right thalamus ( $B=356.4$ ), left inferior frontal gyrus ( $D=26$ ) and left insular gyrus ( $D_{IBS}=28$ ). For HCs, left precuneus ( $B=366.1$ ) and bilateral hippocampus ( $B_{right}=389.1$ ,  $B_{left}=409.5$ ) were considered *hub* regions. The right insular gyrus ( $D_{IBS}=27$ ) was also identified as a *hub* in HCs and the right hippocampus ( $B=409.5$ ) was identified as a *hub* in IBS. Figure 7 depicts the IBS and HC structural networks based on within group *betweenness* metrics for each region.

#### 4.1.1. Discussion

In the current study, we compared regional GM volume from 82 well phenotyped, predominantly premenopausal female IBS and 119 HC subjects, by combining structural imaging data from multiple studies performed at UCLA. We found: 1) Lower GM volume were identified in insula, cingulate, amygdala, hippocampus, putamen and frontal regions, while greater GMV was observed in S1 for IBS compared to HC. 2) Many of the differences were accounted for by a history of EALs but not trait anxiety. 3) Similarities and differences between the two groups in global and regional network characteristics based on regional GM volume changes were observed. To our knowledge, this is the first report of structural brain network alterations in IBS, and the largest analysis of regional GM volume changes for any pain disorder.

#### 4.2.0. Novelty of combining and analyzing data from individual studies

We demonstrated widespread alterations in GM volume in a large number of IBS patients from 7 individual studies, and changes in identified regions were largely consistent with those reported in previous studies. Aggregating data across multiple studies and sites through mega-analyses has accelerated progress in mapping brain structure and function and improved on the limitations of current meta-analytic efforts by increasing statistical power, reliability of findings, representativeness of samples, and the ability to study the causes of variability across various experiments [29]. Although between-study factors may be a source of variability, results that generalize over multiple studies are considered more reliable and less likely to be related to the characteristics of a single study's design [29].

#### 4.3.0. Significant GM volume changes between IBS and HCs

Compared to HCs, IBS showed moderate effect size *reductions* (Cohen's  $d=.30$  to  $.61$ ) in a range of brain regions including bilateral insula, bilateral superior frontal gyrus, bilateral middle orbital frontal gyrus, bilateral hippocampus, bilateral amygdala, left cingulate gyrus, left gyrus rectus, left putamen and brainstem. In contrast, *higher* GM volume in IBS was

only observed for S1. The identified regions with lower GM volumes (including insula, amygdala, cingulate, hippocampus, brainstem, prefrontal modulatory regions and the basal ganglia) are similar to previously reported GM [12; 35; 116] and white matter [25] changes in smaller samples of IBS patients, in some studies with other chronic pain conditions including chronic lower back pain, fibromyalgia, migraine, chronic tension type headache, migraine, chronic facial pain, chronic pain in hip osteoarthritis and chronic regional pain syndrome [89], and patients with affective disorders [13; 63; 74]. However, significant differences to previously published studies in IBS patient populations were also observed. In particular the studies differed in brain regions that showed *higher* GM, with one showing higher GM volume in the hypothalamus [12], and the other, OFG and a trend in posterior INS/S2 cortex [116]. These differences may be due to differences in patient characteristics, use of different statistical algorithms and templates, increased power in the present study to detect moderate effect size differences, rigorous error control applied in the present study, and decreases in variability in the sampling mean estimates afforded by increased sample size.

The current study demonstrates significantly higher GM volume in IBS in S1, a brain region involved in sensory discriminative processing of both noxious and innocuous stimuli [68–70]. GM differences in S1 have been reported both in different chronic pain conditions [33; 38; 53; 54; 71; 94] and in HCs with greater somatic pain sensitivity [44]. For example, in HCs without any pain history a positive correlation between CT in S1 and pain and temperature sensitivity, and a negative correlation of temperature sensitivity with CT in the anterior midcingulate cortex has been reported [44]. Also, in HCs GM increases in response to repetitive noxious stimulation in S1 and ACC have recently been reported [129]. It has been suggested that the critical factor for S1 to undergo functional and structural reorganization maybe the presence of constant sensory input to this brain region [53]. While the basis for such ongoing input to S1 in IBS patients remains unknown, such signals may arise from gut microbial factors [96] from mucosal immune activation [119] or from increased spinothalamic input secondary to alterations in descending pain modulation systems [21]. Consistent with extensive alterations in the somatosensory system are symptom reports [23], and results from quantitative pain testing [104; 125] showing widespread somatic hyperalgesia in IBS patients, including heat pain stimuli. In addition, a recent DTI study has identified widespread microstructural changes in sensory processing and modulation areas of the brain [43].

The mechanisms underlying the observed GM alterations remain to be determined, even though various molecular mechanisms have been proposed [10; 82; 109]. Ultimately, it is unclear whether the observed changes are a consequence of having a chronic pain disorder [34; 89], or if they are underlying, possibly genetically/epigenetically determined risk factors. The fact that lower prefrontal, cingulate, insula, left insula, putamen and hippocampal GM volumes were no longer observed after correction for EALs suggests that these changes may not be specific for IBS per se, and are more related to EALs often reported by IBS patients, and other patients with persistent pain disorders [3; 14; 101]. Lower GM volumes in hippocampus, amygdala, PFC, anterior cingulate and basal ganglia have previously been reported in subjects with a history of EAL [22; 28; 56; 65]. Such EAL-

related structural brain changes may be the consequence of epigenetic modifications of gene expression, including glucocorticoid receptor methylation and resulting chronically elevated plasma cortisol levels [22]. The small correlations identified between frontal brain regions and abdominal pain, fronto-insular regions and symptom severity, and between the left inferior frontal gyrus and symptom duration suggest possible correlations between the observed structural changes and IBS symptoms, but larger samples are needed to replicate these exploratory observations.

#### 4.4.0. Network analyses

No statistically significant group differences were found for network indices of global or local efficiency of information transfer between regions. Consistent with previous studies examining GM morphometry in HCs [7; 58] and migraine patients [85], the global organization of structural networks based on GM volume in HC subjects as well as IBS showed small world properties, which are thought to provide the optimal balance between network segregation and integration resulting in economical brain functioning that minimizes wiring cost and supports dynamic complexity [7; 19].

With regard to regional network properties, several group differences were observed potentially reflecting disease-related alterations in brain regions that are critical in facilitating global integrative processes and communication. Compared to HCs, the right cingulate gyrus and right thalamus, were found to be more critical for controlling information in IBS. This greater *betweenness* has also been reported for anterior cingulate, midcingulate and thalamic GM volumes in female migraine patients compared to HCs [85]. This lends support to the view that other often comorbid pain conditions may have similar underlying brain network alterations which affect the processing and modulation of sensory information [32; 72; 126].

The regions identified as unique hubs in IBS are regions shown in functional MRI studies to be involved in endogenous pain modulation and central sensory amplification including left inferior frontal cortex, left insular cortex, right thalamus and brainstem [137]. While the observed alterations in regional topology provide strong evidence for extensive structural reorganization of cortical and subcortical regions previously implicated in altered brain responses to visceral pain stimuli and their expectation [55; 87; 133], the current study does not allow us to implicate specific physiological or molecular mechanisms underlying these changes.

#### 4.5.0. Study limitations

Our method of data aggregation is similar to other recent efforts to gain power by combining multi-site data [11]. Although the current study was adequately powered to detect medium effect size volumetric differences, small effect size differences cannot be ruled out. Although, we found no differences in TGM or TBV by acquisition protocol, we cannot rule out variance due to acquisition parameters. Due to the nature of the experimental design, variability accounted for by specific and nonspecific study effects and acquisition protocols is confounded and cannot be separated. Unfortunately, the size of some of the LBPA 40 atlas template regions limited our ability to parse out specific subregions of the insula,

cingulate cortex, and prefrontal cortex [19]. With regard to the network analysis, inter-regional volumetric connectivity does not necessarily reflect anatomical (i.e., white matter tracts) or functional connectivity, although moderate approximations have been reported [52; 64; 81]. Our findings about basic network properties and some specific alterations are consistent with previously reported regional structural and functional alterations,[34] including alterations in network properties in chronic pain conditions[85], and suggests structural alterations in brain networks involved in sensory processing and modulation. However, the correlation of the observed network alterations based on grey matter changes with structural and functional network alterations based on other imaging and analysis modalities (including probabilistic tractography and resting state networks), as well as the clinical consequences of such network alterations remain to be determined [16].

#### 4.6.0. Summary and conclusions

The observed changes (increases and decreases) in GM volume and alterations in regional network properties identified in this study may reflect different pathophysiological components of the disease processes underlying IBS symptoms, including increased sensitivity to somatic and visceral stimuli (higher GM volumes in S1), associated increase in emotional arousal (lower GM in hippocampus in IBS) and other chronic pain related mechanisms (lower GM in insula and cingulate cortices, differences in insula, cingulate, thalamus and brain stem network properties). Future studies in large well phenotyped samples of patients, as well as in animal models of chronic pain will be required to address these hypotheses.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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### Abbreviations

<b>IBS</b>	Irritable bowel syndrome
<b>HCs</b>	healthy controls
<b>GM</b>	gray matter
<b>VBM</b>	voxel-based morphometry
<b>CT</b>	cortical thickness
<b>ROI</b>	region of interest
<b>S1</b>	somatosensory cortex

<b>ACC</b>	anterior cingulate cortex
<b>mOFG</b>	middle orbitofrontal gyrus
<b>PFC</b>	prefrontal cortex
<b>HPA</b>	hypothalamic pituitary adrenal
<b>LONI</b>	Laboratory of Neuroimaging
<b>GI</b>	gastroenterology
<b>EALs</b>	early adverse life events
<b>ETI</b>	Early Life Trauma Inventory
<b>STAI</b>	State Trait Anxiety Inventory
<b>HAD</b>	Hospital Anxiety and Depression Scale
<b>CSQ</b>	Coping Strategies Questionnaire
<b>PHQ</b>	Patient Health Questionnaire
<b>BSQ</b>	Bowel Symptoms Questionnaire
<b>GLM</b>	general linear model
<b>L</b>	left
<b>R</b>	right
<b>C</b>	clustering coefficient
<b>D</b>	degree
<b>B</b>	betweenness

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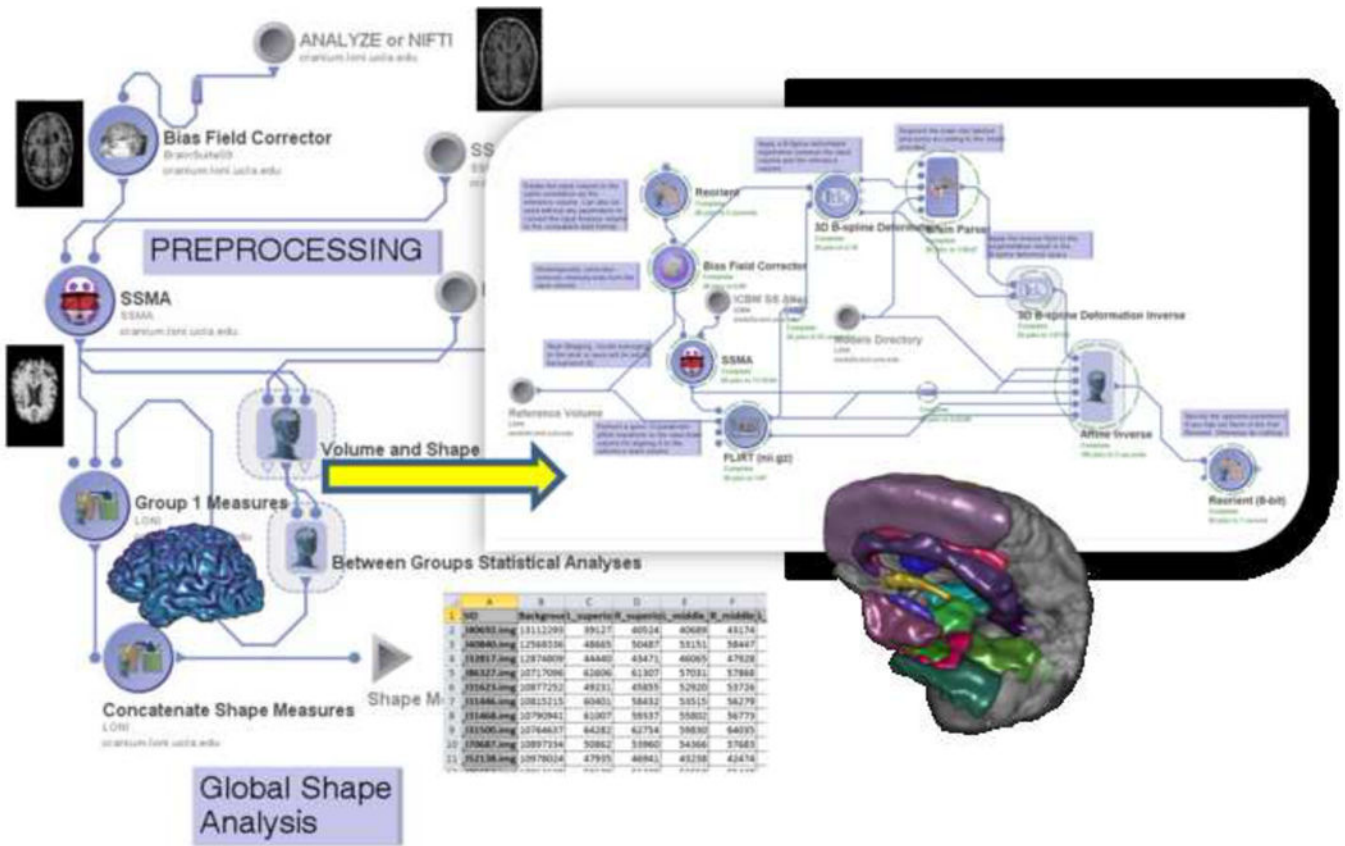
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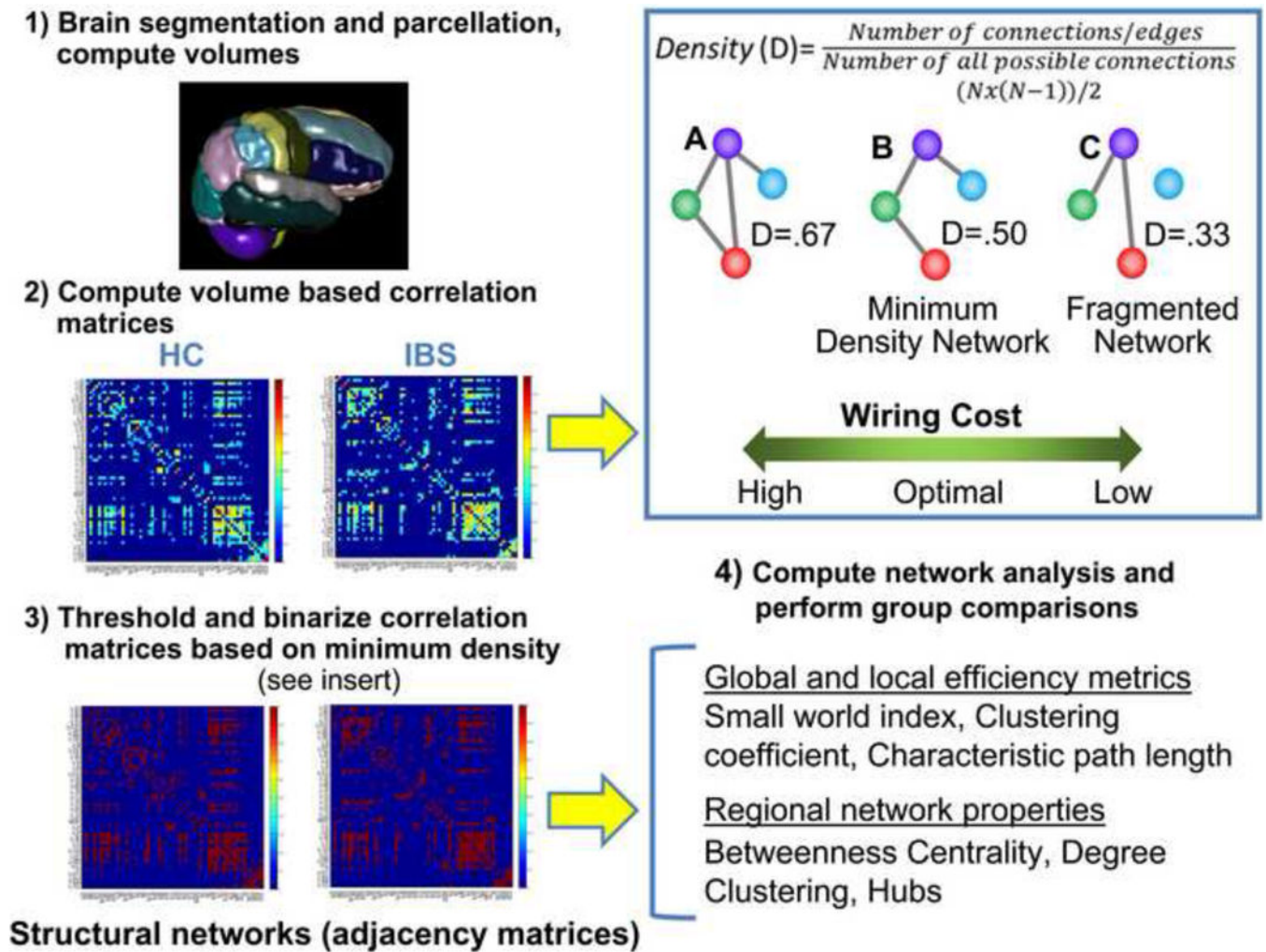


**Figure 1.**

Volumetric pipeline workflow.

This workflow represents a heterogeneous computational protocol that includes data preprocessing (inhomogeneity correction, skull stripping), brain parcellation, volumetric calculation and statistical analysis of derived neuroimaging biomarkers (i.e., volume) and various subject phenotypes (e.g., diagnosis, age). The insert image illustrates part of the nested processing modules part of the volume and shape processing step of the protocol. Examples of outputs of key processing modules are shown as thumbnail images throughout the workflow. The nodes in the pipeline workflow graph represent atomic processing tools or nested groups of data analysis modules. The edges connecting different nodes in the pipeline graph indicate the data flow and the dependencies of the execution protocol. The volumetric workflow pipeline consists of 4 main components (each annotated by a label) – data preprocessing (intensity inhomogeneity correction and skull-stripping, cortical surface modeling, and tissue classification).

Abbreviations : SSMA- skull-stripping meta-algorithm.

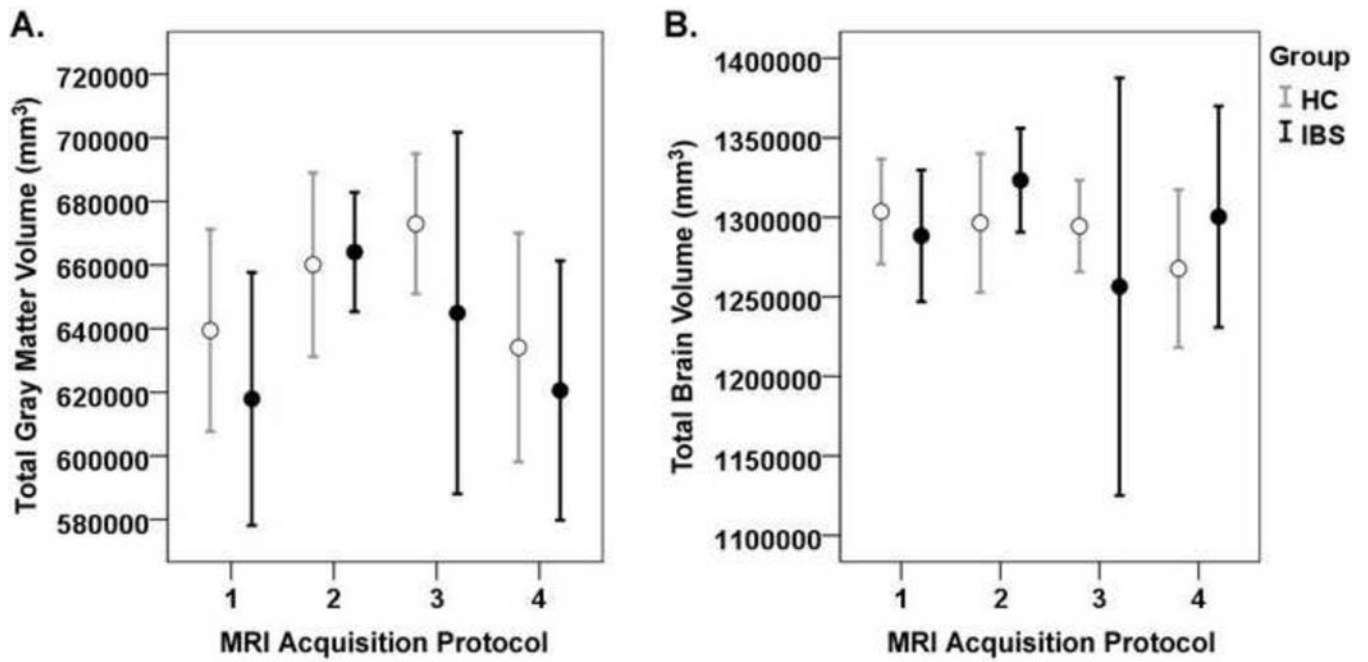


**Figure 2.**  
Network analysis pipeline.

The pipeline consisted of 1) Compute volumes for 62 regions for each subject based on subject-specific brain parcellations using the LPBA 40 and Harvard-Oxford atlases. 2) After controlling for total brain volume with linear regression, inter-regional volume based correlation matrices were computed for each group. 3) Structural brain networks also known as adjacency matrices were then constructed by binarizing the correlation matrices based on the minimum network density threshold (see insert). 4) Based on group adjacency matrices global and regional network metrics and statistical analysis of group differences was performed. Inset. Density is the ratio of the number of connections present to the number of possible connections and is a measure of wiring cost.

Here we present three networks (A,B,C) with varying densities in the context of wiring cost. A minimum density network is a network where all the regions or nodes of the network are fully connected that is, each region has at least one connection). Wiring in the brain is thought to be based on the principle of wiring minimization and cost efficiency which permits the most economical use of space and metabolic costs in the brain. Greater wiring costs (more connections) are associated with greater density (A). Minimum density (B)

reflects the minimum wiring cost possible before network fragmentation ( $C$ ). The minimum density network is considered to have optimal wiring cost. Abbrev  $N=$  connection also called an edge between two regions.

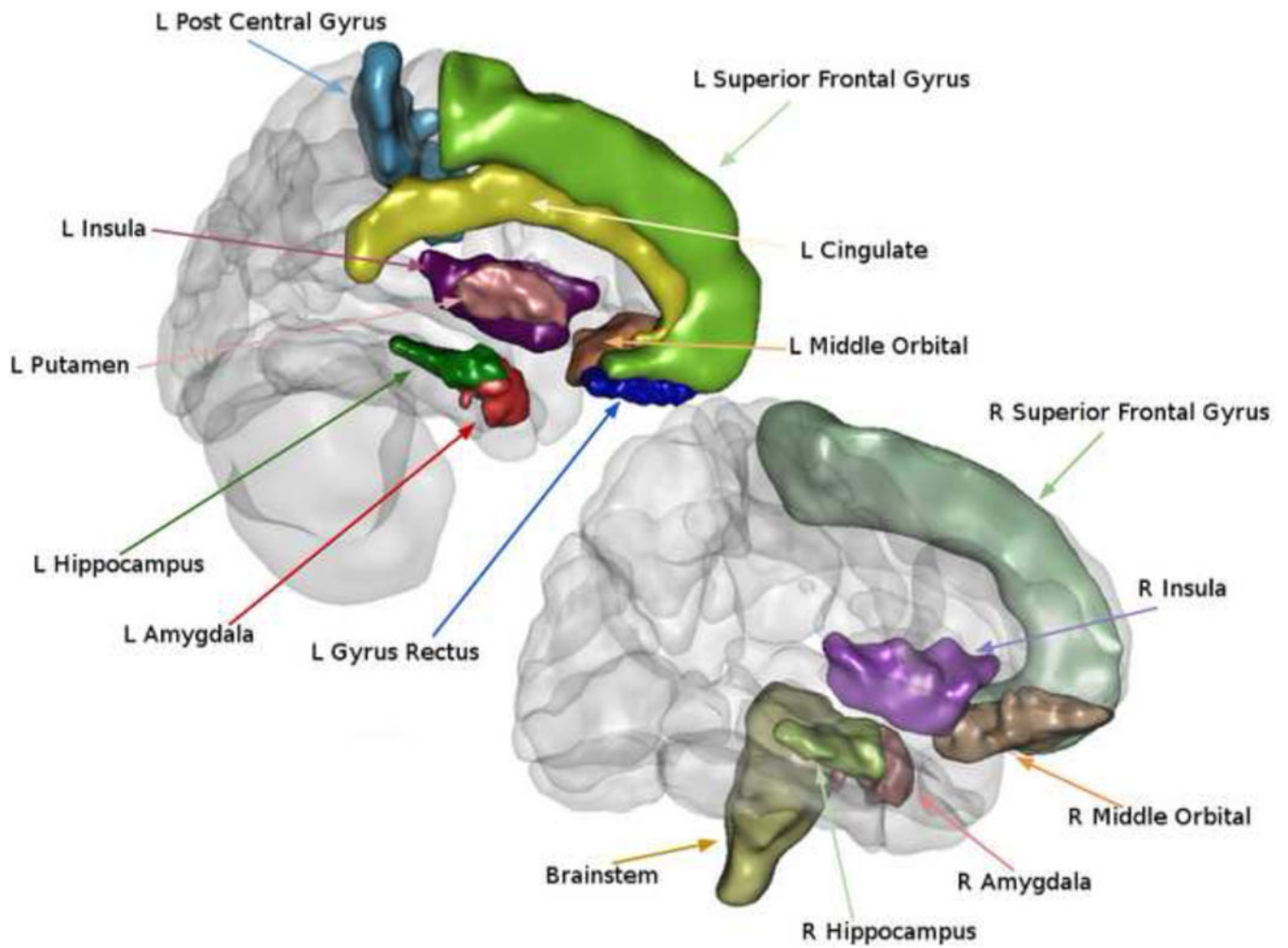


**Figure 3.**

Gray matter volume by study protocol.

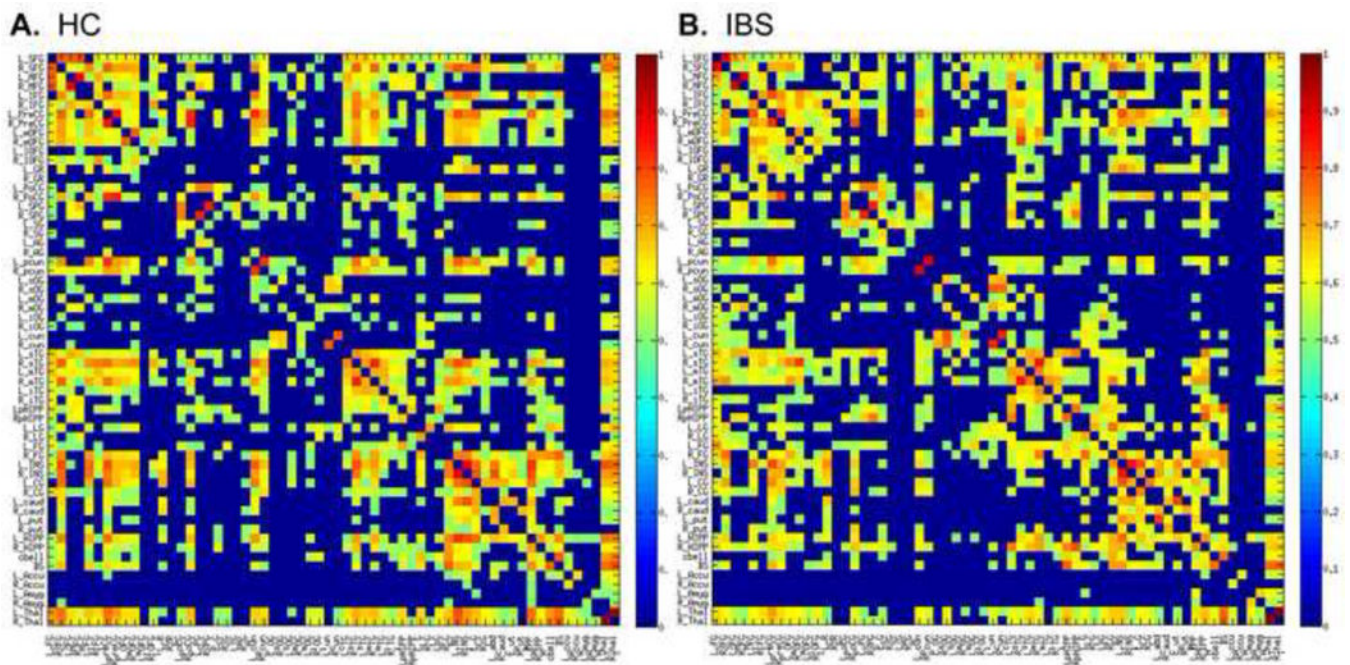
These graphs depicts the mean total grey matter (A) and mean total brain volume (B) and ±95% confidence intervals by acquisition protocol (protocols 1, 2, 3, 4) and group (healthy controls (HCs) and irritable bowel syndrome patients (IBS)). Details of the protocols are described in Table 4.





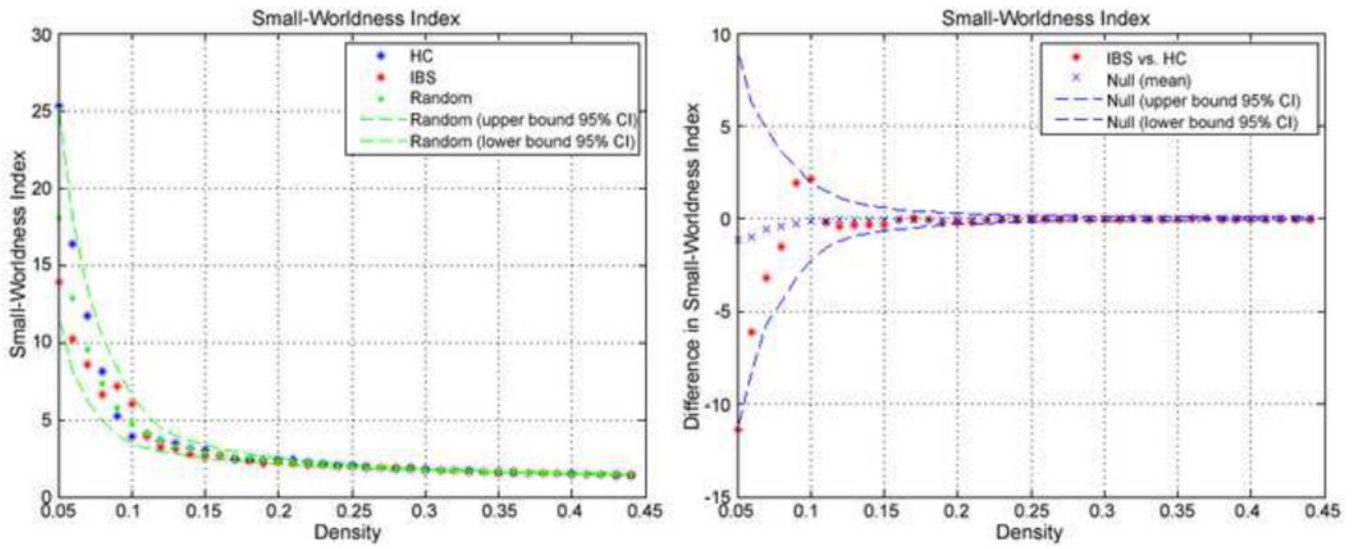
**Figure 4.** Regions showing volumetric differences between healthy controls and irritable bowel syndrome patients. The atlas-based ROIs that showed group differences are illustrated. ROIs were determined using the LONI probabilistic brain atlas (LBPA 40) and the Harvard-Oxford Atlas.



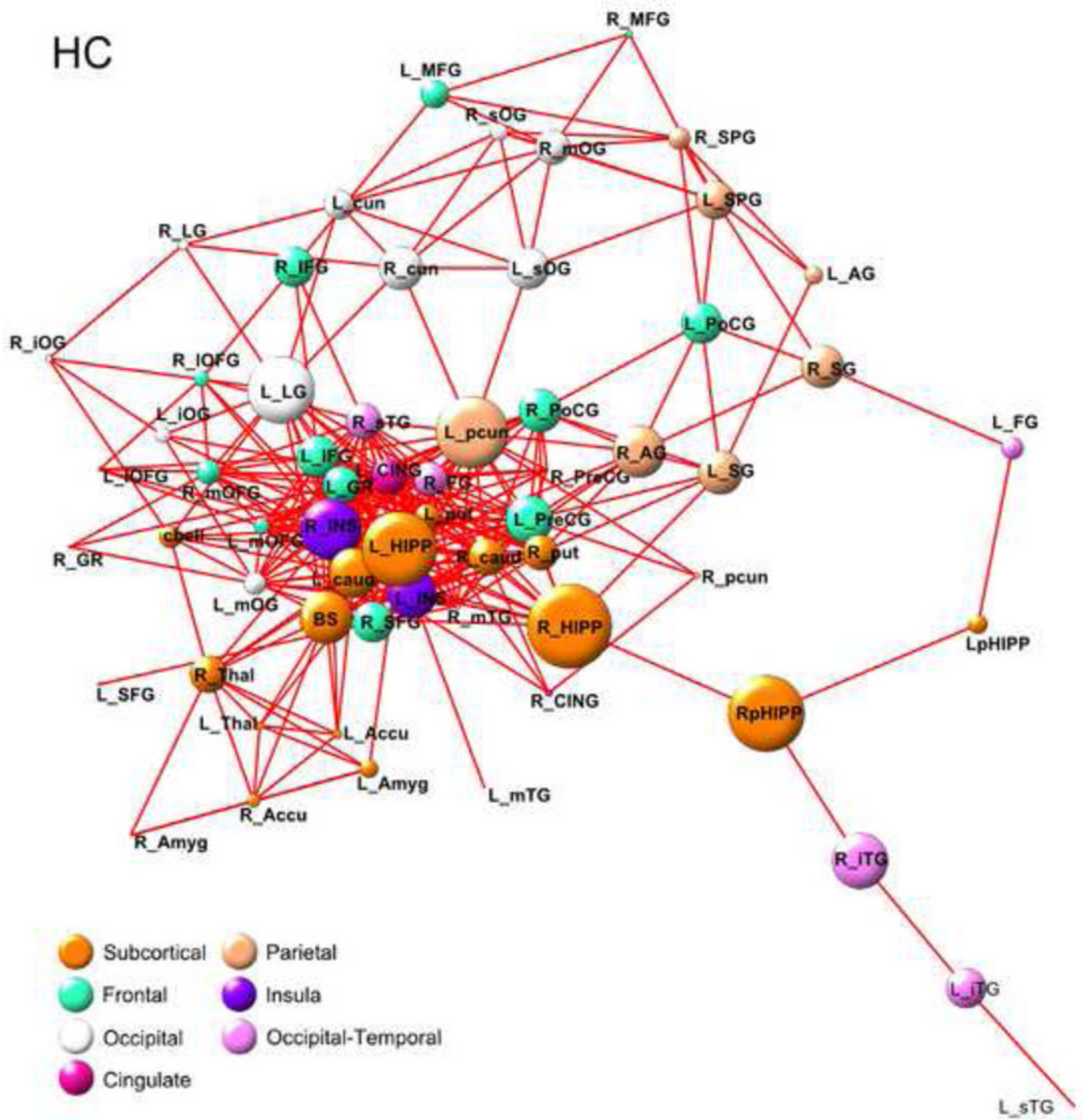


**Figure 5.**

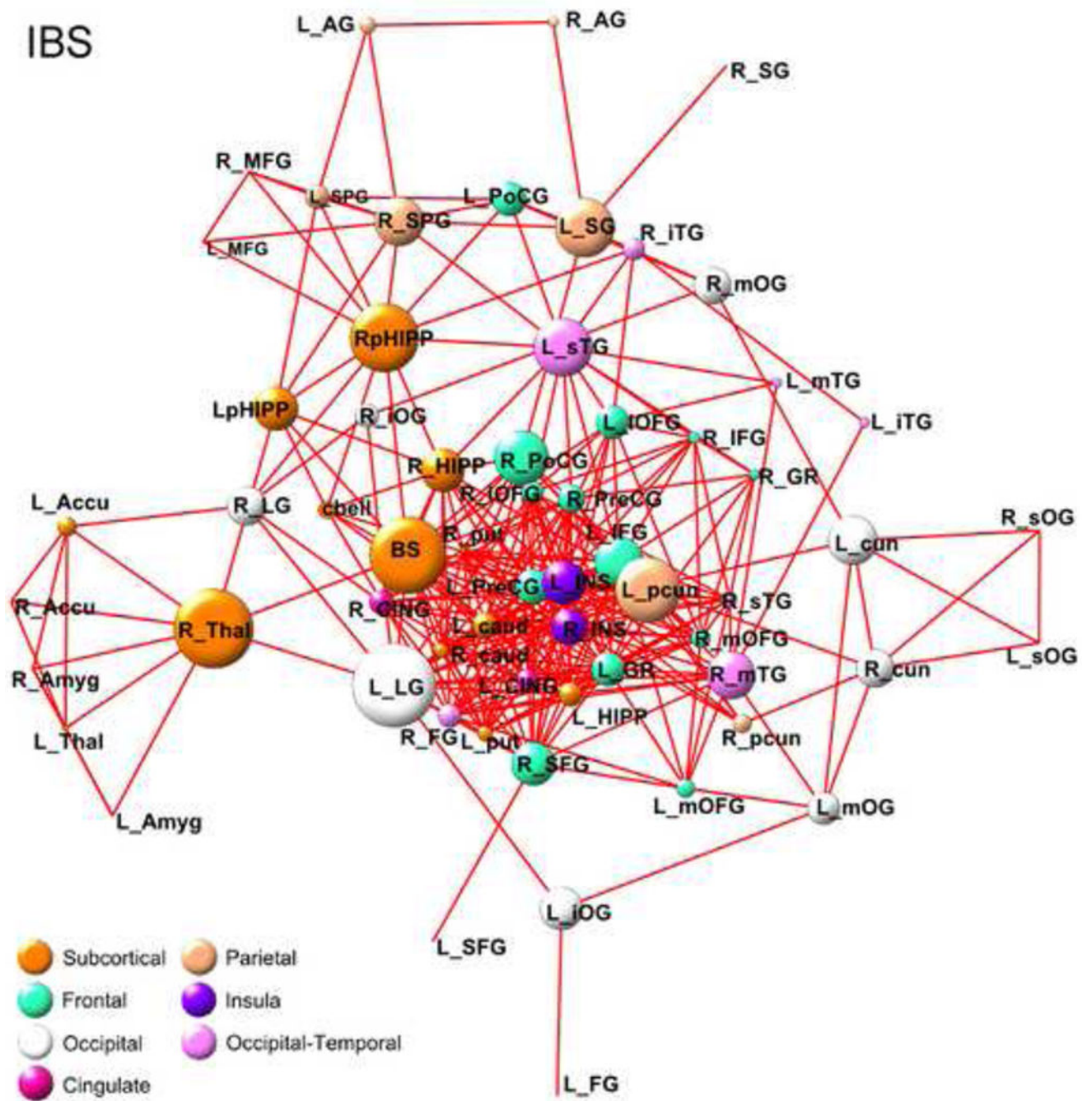
Thresholded inter-regional correlation matrices. Each colored box represents a correlation between specified brain regions. Abbreviations for brain regions are defined in Table 3. Group specific inter-regional correlation matrices for 62 regional gray matter volumes were thresholded at the minimum density (17%) by setting correlation values to zero where  $r < .32$  for irritable bowel syndrome (IBS) and  $r < .25$  for healthy controls (HC). In addition, all values across the diagonal of the matrices, representing the correlation of a region with itself were set to zero. Correlation values range from 0 (dark blue) to 1 (dark red).



**Figure 6.** Global network properties of volumetric networks. A) Small world indices across a range of densities. B) Between-group differences and 95% confidence intervals in small-world index as a function of density. Positive values indicate  $IBS > HC$  and negative values indicate  $IBS < HC$ .







**Figure 7.** HC and IBS betweenness-based structural network energized using a Kamada-Kawai algorithm. Betweenness is a proportional measure of how often a node (brain region) is located on the shortest path between two brain regions, where shortest path refers smallest distance between two vertices and corresponds to the length (number of edges) between them. Here,

the greater betweenness of a region is depicted by larger circle magnitudes. Edges of the graph represent connected regions. Abbreviations for brain regions are defined in Table 3

**Table 1**

**Structural MRI acquisition protocols**

Brain images were obtained from 201 females from 7 structural imaging studies conducted at UCLA using 4 protocols. Abbreviations: HC-healthy control, IBS-irritable bowel syndrome, TR- repetition time, TE- echo time, MRI-magnetic resonance imaging.

	HC (n)	IBS (n)	Age years m(SD) range	Field Strength	TR (ms)	TE (ms)	Flip Angle (°)	Number of Scans
<b>Protocol 1</b>	24	20	29 (11.2) 18 – 64	3 Tesla	20	3.39	25	44
<b>Protocol 2</b>	26	45	31(9.3) 19 – 56	3 Tesla	2300	2.85	9	71
<b>Protocol 3</b>	50	8	32 (9.7) 20 – 56	1.5 Tesla	1900	4.38	15	58
<b>Protocol 4</b>	19	9	30(10.9) 19 – 61	1.5, 3 Tesla	2200	3.26	9	28



**Table 2**

Distribution of acquisition protocols and subjects across studies.

study	protocol	Subjects
1	1	26
2	1,2,3	14
3	1,2,3,4	108
4	1,2,3,4	25
5	2,4	16
6	4	7
7	2,3	5

**Table 3**  
**Cortical and subcortical regions and corresponding abbreviations based on LONI probabilistic brain atlas (LBPA 40) and the Harvard-Oxford atlas**

Abbreviations L-left, R-right.

<b>Frontal Lobe</b>		<b>Occipital Lobe</b>	
L superior frontal gyrus	L_SFG	L superior occipital gyrus	L_sOG
R superior frontal gyrus	R_SFG	R superior occipital gyrus	R_sOG
L middle frontal gyrus	L_MFG	L middle occipital gyrus	L_mOG
R middle frontal gyrus	R_MFG	R middle occipital gyrus	R_mOG
L inferior frontal gyrus	L_IFG	L inferior occipital gyrus	L_iOG
R inferior frontal gyrus	R_IFG	R inferior occipital gyrus	R_iOG
L precentral gyrus	L_PreCG	L cuneus	L_cun
R precentral gyrus	R_PreCG	R cuneus	R_cun
L middle orbitofrontal gyrus	L_mOFG		
R middle orbitofrontal gyrus	R_mOFG	<b>Temporal Lobe</b>	
L lateral orbitofrontal gyrus	L_IOFG	L superior temporal gyrus	L_sTG
R lateral orbitofrontal gyrus	R_IOFG	R superior temporal gyrus	R_sTG
L gyrus rectus	L_GR	L middle temporal gyrus	L_mTG
R gyrus rectus	R_GR	R middle temporal gyrus	R_mTG
		L inferior temporal gyrus	L_iTG
<b>Parietal Lobe</b>		R inferior temporal gyrus	R_iTG
L postcentral gyrus	L_PoCG	L parahippocampal gyrus	L_pHIPP
R postcentral gyrus	R_PoCG	R para hippocampal gyrus	R_pHIPP
L superior parietal gyrus	L_SPG	L lingual gyrus	L_LG
R superior parietal gyrus	R_SPG	R lingual gyrus	R_LG
L supramarginal gyrus	L_SG	L fusiform gyrus	L_FG
R supramarginal gyrus	R_SG	R fusiform gyrus	R_FG
L angular gyrus	L_AG		
R angular gyrus	R_AG	<b>Limbic Lobe</b>	
L precuneus	L_pcn	L cingulate gyrus	L_Cing
R precuneus	R_pcn	R cingulate gyrus	R_Cing
		L hippocampus	L_HIPP
<b>Other Structures</b>		R hippocampus	R_HIPP
L insular cortex	L_INS		
R insular cortex	R_INS	<b>Subcortical (Harvard-Oxford Atlas)</b>	
L caudate	L_caud	L Thalamus	L_THAL
R caudate	R_caud	R Thalamus	R_THAL
L putamen	L_put	L Amygdala	L_Amyg
R putamen	R_put	R Amygdala	R_Amyg
Cerebellum	cbell	L Nucleus Accumbens	L_Accu

<b>Frontal Lobe</b>		<b>Occipital Lobe</b>	
Brainstem	BS	R Nucleus Accumbens	R_Accu

**Table 4**

**Demographic and behavioral variables**

Mean and standard deviation (SD) for healthy controls (HC) and irritable bowel syndrome (IBS) patients. Patient health questionnaire was score without gastrointestinal items.

	Group	Sample Size	Mean (SD)	Independent sample t-test (df)	p value
Age	HC	119	29.9 (10.3)	-1.59 (199)	.12
	IBS	82	32.2 (9.6)		
Early Trauma Inventory- Global	HC	107	3.3 (3.7)	-1.72 (183)	.09
	IBS	78	4.2 (3.7)		
Anxiety	HC	116	3.8 (3.1)	-3.33 (192)	.001
	IBS	78	5.4 (3.3)		
Depression	HC	116	1.6 (2.3)	-2.67 (192)	.008
	IBS	78	2.6 (2.5)		
Patient Health Questionnaire	HC	76	2.4 (2.0)	-6.99 (121)	$1.6 \times 10^{-6}$
	IBS	47	5.8 (3.3)		
Trait Anxiety	HC	119	45.5 (9.8)	-4.05 (197)	.00007
	IBS	80	51.1 (9.6)		
Catastrophizing	HC	92	0.4 (0.6)	-6.63 (166)	$4.4 \times 10^{-10}$
	IBS	76	1.2 (1.0)		

**Table 5**  
**Clinical and symptom measures in irritable bowel syndrome (IBS)**

Mean and standard deviation (SD) for 82 IBS patients. Information on bowel habit was missing for one subject.

	Sample Size	Mean (SD)
Overall symptom severity past week	75	10.9(4.4)
Abdominal pain past week	77	9.5(4.9)
Usual symptom severity	81	3.2(0.59)
Duration	76	12.7(8.9)

	Frequency Number
Constipation	31(38%)
Diarrhea	22 (27%)
Alternate	16 (20%)
Unspecified	6 (7.0%)
Mixed	5 (6%)

**Table 6**  
**Brain regions demonstrating volumetric group differences comparing HC-IBS controlling for total brain volume**

F statistics and q value along with standardized effect size, Cohen's d, are reported first from the linear contrast analysis (HC-IBS) on the mean volumetric estimates from the general linear model controlling for total brain volume. Due to the direction of the contrast positive effect sizes represent greater volumes in HCs compared to IBS and negative effect sizes represent greater volume in IBS patients. The last two columns of the table indicate results from linear contrasts analyses after adding the square root of trait anxiety and the square root of global early life trauma scores as covariates to the general linear model controlling for TBV. We report whether group differences in regional volume persisted as indicated by q<.05. Y=denotes regional significance (q<.05) after covariate analysis, N denotes lack of significance. It should be noted that neither trait anxiety of early life trauma scores were considered statistically significant covariates (q<.05) but accounted for enough variance to have effect in the models.  
 Abbreviations: healthy control- HC, IBS –irritable bowel syndrome, FDR-false discovery rate, SE-standard error

Region of Interest	F (1,198)	FDR Adjusted p q	Mean (mm <sup>3</sup> )	SE	Mean (mm <sup>3</sup> )	SE	Cohen's d	Group differences remain after controlling for trait anxiety	Group differences remain after controlling for early life trauma
L superior frontal gyrus	4.3	0.029	51401.4	307.0	50407.2	370.0	0.30	Y	N
R superior frontal gyrus	8.5	0.006	54705.5	440.1	52689.6	530.3	0.42	Y	N
L cingulate	9.5	0.006	11358.7	142.1	10673.0	171.3	0.44	Y	N
Braunstem	4.9	0.024	26649.4	255.1	25766.7	307.5	0.32	N	N
L insula	7.6	0.008	8310.9	101.3	7874.0	122.1	0.40	Y	N
R insula	10.3	0.006	5510.5	87.1	5071.9	105.0	0.46	Y	N
L middle orbital frontal gyrus	6.5	0.012	8618.3	71.0	8335.4	85.6	0.37	Y	N
R middle orbital frontal gyrus	5.2	0.021	9361.6	84.0	9060.3	101.2	0.33	Y	N
L gyrus rectus	7.9	0.007	2078.9	40.8	1898.9	49.2	0.61	Y	N
L hippocampus	8.6	0.006	3674.8	37.5	3502.0	45.2	0.42	Y	N
R hippocampus	8.5	0.006	4008.6	30.7	3868.9	36.9	0.42	Y	N
L Putamen	6.8	0.011	2909.1	59.5	2666.5	71.7	0.38	Y	N
L amygdala	4.4	0.028	1224.9	24.0	1145.4	28.9	0.31	Y	N
R amygdala	9.3	0.006	1219.8	25.7	1096.7	31.0	0.44	Y	Y
L post central gyrus	14.1	0.003	20134.9	220.3	21431.5	265.5	-0.54	Y	Y



**Table 7**  
**Network metrics at minimum density**

Global network measures for healthy control (HC) and irritable bowel syndrome (IBS) structural networks at the minimum density =.17.

	HC	IBS	pval
Mean Degree	11.0	11.0	0.99
Mean Clustering Coefficient	0.57	0.52	0.38
Normalized Clustering Coefficient	3.23	3.00	0.38
Global Efficiency	0.49	0.50	0.99
Mean Local Efficiency	0.70	0.64	0.43
Modularity	0.28	0.28	0.36
Characteristic Path Length	2.55	2.42	0.20
Normalized Path Length	1.34	1.27	0.20
Mean Node Betweenness	97.2	88.8	0.26
Mean Edge Betweenness	2.55	2.42	0.30
Small Worldliness	2.41	2.36	0.20