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## Altered Brain Structure and Functional Connectivity and its Relation to Pain Perception in Girls with Irritable Bowel Syndrome

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### Abstract

**Objective:** Imaging studies in adults with IBS have shown both morphological and resting state (RS) functional connectivity (FC) alterations related to cortical modulation of sensory processing. Since analogous differences have not been adequately investigated in children, this study compared gray matter volume (GMV) and RS-FC between girls with IBS and healthy controls (HC), and tested the correlation between brain metrics and laboratory-based pain thresholds (Pth).

**Methods:** Girls with Rome III criteria IBS (N=32) and matched HCs (N=26) were recruited. In a subset of patients, Pth were determined using a thermode to the forearm. Structural and RS scans were acquired. A voxel-based general linear model, adjusting for age, was applied to compare differences between groups. Seeds were selected from regions with group GMV differences for a seed-to-voxel whole-brain RS-FC analysis. Significance for analyses were considered at  $p < .05$  after controlling for false discovery rate. Significant group differences were correlated with Pth.

**Results:** Girls with IBS had lower GMV in the thalamus, caudate nucleus (CaN), nucleus accumbens, anterior mid-cingulate (aMCC), and dorsolateral prefrontal cortex (dlPFC). They also exhibited lower RS-FC between the aMCC and the precuneus, but greater connectivity between

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the CaN and precentral gyrus. Girls with IBS had higher Pth with a moderate effect size ( $t_{(22.81)} = 1.63$ ,  $p = 0.12$ ,  $d = 0.64$ ) and lower thalamic GMV bilaterally was correlated with higher Pth (left:  $r = -.62$ ,  $p_{(FDR)} = .008$ ; right:  $r = -.51$ ,  $p_{(FDR)} = .08$ ).

**Conclusion:** Girls with IBS had lower GMV in the PFC, basal ganglia, and aMCC, as well as altered functional connectivity between multiple brain networks, suggesting that structural changes related to IBS occur early in brain development. Girls with IBS also showed altered relationships between pain sensitivity and brain structure.

### Keywords

children with irritable bowel syndrome; pain sensitivity; sensory processing; cortical modulation; functional and structural MRI

## Introduction

Irritable Bowel Syndrome (IBS) is a functional disorder of the brain gut-axis characterized by recurrent abdominal pain and altered bowel habits that are not explained by structural or biochemical abnormalities. Although more frequently diagnosed in adults than in children, IBS is the most common cause of functional Recurrent Abdominal Pain (RAP) in children in the Western Hemisphere (1). A recent study by Hyams et al (2) found in their sample of 507 participants, up to 17% of adolescents with abdominal pain fit criteria for IBS. El-Matery et al (3) found that 36% of their child sample originally presenting with RAP fit the criteria for IBS. IBS symptoms can impair functioning in daily life (4) and can persist into adulthood (5).

The pathophysiology of IBS can be characterized as a bidirectional brain-gut axis disorder. Multiple brain networks, including the salience, sensorimotor and executive-control networks, are thought to mediate the effects of affect, mood and environmental factors on gut function and pain perception, resulting in visceral hypersensitivity and altered bowel habits (6,7). For a review on these networks see (8). Prefrontal brain regions modulate activity in the paralimbic regions, subregions of the anterior cingulate cortex (ACC) and hypothalamus, which then in turn modulate activity of descending pathways through the periaqueductal gray and brainstem nuclei. Activity in these corticolimbic pontine networks mediates the effects of cognitions and emotions on the perception of visceral pain and discomfort (6). The enteric nervous system and the brain are engaged in bi-directional communication in these pathways through vagal and sympathetic fibers. Alterations within this nervous system, whether it be in the gut (bottom-up) or brain (top-down), can result in IBS symptoms (8). These brain network abnormalities are thought to lead to disturbances in the brain in areas related to affect such as the cingulate, amygdala (9) and prefrontal regions (10). Moreover, as many prefrontal regions are involved in central pain inhibition (11), abnormalities in these areas due to chronic IBS may lead to deficiencies in the ability to engage in the endogenous modulation of pain and altered pain sensitivity.

Past research in adult patients with IBS and pain sensitivity have shown mixed results, including *hyperalgesia*, to mechanical and thermal pain (12–14) and somatic *hypoalgesia* at non-pain sites such as the hand (15–18). In children, mixed results regarding thermal pain

thresholds have also been observed (19–22), including *hypoalgesia* in response to thermal pain at the hand (20). Studies investigating central mechanisms and changes in pain sensitivity via imaging modalities in IBS have been carried out in adults (23–26). Common brain regions engaged during pain stimuli in adults with IBS relative to controls have been the insula (**INS**), anterior cingulate cortex (**ACC**), primary somatosensory cortex (**S1**), prefrontal cortex (**PFC**), posterior parietal cortex (**PPC**), and thalamus (**THAL**) (27–30). Additionally, patients with IBS have shown activation in the emotional-arousal network (ACC and amygdala) and midbrain in response to visceral pain stimulation, a finding not seen in controls (31). Despite all of the research done in adults, we know of only one study using magnetic resonance imaging (MRI) in children with IBS (32).

Hubbard et al. (32) recently found lower cortical thickness in pediatric IBS patients in the dorso-medial prefrontal cortex (**dmPFC**), dorso-lateral prefrontal cortex (**dIPFC**) and PPC, and an increase in the posterior cingulate cortex (**PCC**). It was found that IBS patients exhibited increased connectivity from the dIPFC to cuneus and from the right PCC to the dIPFC. Controls had greater connectivity from the PCC to the parietal operculum (**INS**). These atypical connectivity patterns in nodes responsible for cognitive control (33) are thought to lead to disinhibition of descending pain modulation, attention to viscerosensory processes, and exacerbated visceral hyperalgesia.

The primary aim of the following study was to investigate structural differences in grey matter volume (**GMV**) in the brain using voxel-based morphometry and its associated resting state (**RS**) abnormalities in functional connectivity (**FC**) in girls with IBS compared to healthy, age and sex-matched controls. A secondary aim was to assess if these changes in brain structure and connectivity were associated with differences in state anxiety, trait anxiety, daily pain levels, pain-related anxiety, and pain thresholds. It was hypothesized that, in line with the adult findings, children with IBS would show differences from controls in GMV in regions such as the cingulate, somatosensory cortex, insula, putamen, hippocampus, amygdala, and frontal executive control regions (23). Additionally, we postulated that in an exploratory analysis the brain regions that showed IBS related differences in GMV would show altered resting state functional connectivity (**RS-FC**) as well.

## Materials and Methods

### Participants

Study participants included 32 girls; aged 7–17 years, diagnosed with IBS using Rome III criteria (mean age = 11.40, SD = 3.01) and 26 age and sex matched healthy controls (mean age = 10.72, SD = 2.79). See Table 1 for sample breakdown. Participants with IBS were prospectively recruited from three clinical sites: the UCLA Pediatric Pain Clinic and the UCLA-affiliated Whole Child LA (WCLA) pain clinic by LZ, and the Baylor College of Medicine / Texas Children's Hospital pediatric gastroenterology and primary care clinics by RS. Age-matched healthy controls were recruited via Craigslist, postings around campus, and referrals from other participants. All procedures were approved by the UCLA Medical Institutional Review Board and the Institutional Review Board for Baylor College of Medicine. The parents of all participants provided informed written consent and participants provided assent. Exclusion criteria for both groups included (1) acute illness or injury that

may impact lab performance (e.g., fever) or that affects sensitivity of the extremities (e.g., Reynaud's disease); (2) participants being treated for chronic disease other than pain (e.g., diabetes); (3) daily use of opioids (participants using other analgesics were included, but were asked not to take these analgesics on the day of the lab session); (4) antidepressant use unless the dose had been stable for 3 months or longer; (5) developmental delay, autism, or significant anatomic impairment that may preclude understanding study procedures (e.g. developmental age of less than 7 years, assessed by discussion with parents); (6) significant claustrophobia as assessed by discussion with parents and with children. For the IBS group, diagnosis of an organic GI illness (e.g., celiac disease; inflammatory bowel disease) resulted in exclusion. These exclusion criteria were applied at both sites (UCLA/WCLA & Baylor). Data was collected from April 24, 2013 to November 3, 2015.

### **Laboratory pain testing – UCLA Sample**

Following the collection of psychological and clinical measures, girls at UCLA were connected to a physiological monitoring device for a 10-minute baseline recording. Using a procedure conducted in girls with IBS aged 7–12 years (19), a thermode was placed on the volar surface of the right forearm, and, using a Medoc TSA-II Neurosensory Analyzer (Ramat-Yishai, Israel), the temperature increased from 32° C at a rate of 1.5° C/sec until the participant indicated that the heat was painful. This assessment of heat pain threshold was conducted four times with 15 sec inter-stimulus intervals (ISI) as a baseline. The average number of seconds of the four stimuli was considered the heat pain threshold for each participant. Volar forearm pain thresholds have been frequently used as a proxy measure for the central sensitization that occurs in patients with IBS (7,34) as administering visceral pain testing can be quite invasive. Laboratory pain testing was not available at the Baylor site at the time of the study.

### **MRI Acquisition – UCLA and Baylor Samples**

For the UCLA sample, imaging was completed on a different day following the lab testing. Imaging at both sites was performed on a Siemens 3 Tesla Trio scanner with a 12-channel head coil. After careful positioning of the participant, padding of the head to reduce movement, and applying noise-reducing headphones, a standard high-resolution T1-weighted magnetization-prepared rapid acquisition gradient echo (MP-RAGE) scan was obtained; (TE: 2.98 ms, TR: 2300, flip angle: 9 degrees, FOV: 256 mm × 240 mm, percent phase FOV: 93.75, slice thickness 1.0 mm, voxel size 1 mm<sup>3</sup>, 240 slices), lasting about 6 minutes. Participants viewed an age appropriate video during structural imaging to help reduce movement. After a short break, participants were instructed to lie quietly with their eyes closed for a 10-minute resting state functional scan, utilizing the following parameters: TE: 30 ms, TR: 2000 ms, flip angle: 77 degrees, FOV 220mm × 220 mm, slice thickness 4.0mm with a 0.5mm skip, acquisition matrix 64 × 64. 31 axial/oblique slices are obtained, with whole-brain coverage. UCLA and Baylor sites utilized the same MRI acquisition parameters.

## Psychological and Clinical Measures

At the UCLA site, a Numerical Rating Scale (**NRS**), a reliable and valid measure in children 8 years and older (35), assessed current levels of abdominal pain and state anticipatory anxiety for the lab testing and imaging procedures. Ratings were made using a 0–10 scale where 0 = no pain/anxiety and 10 = worst pain/anxiety. The State-Trait Anxiety Questionnaire (**STAI**) was used to assess state (20 items,  $\alpha = .88$ ) and trait (20 items,  $\alpha = .91$ ) anxiety (36) in the UCLA and Baylor samples. The Child Pain Anxiety Symptoms Scale (**CPASS**), a reliable and valid measure of pain-related trait anxiety, was assessed only in the UCLA child sample (20 items,  $\alpha = .90$ ), ages 8–18 years (37). As these measures were not collected during the brain imaging procedure, results associating these two different procedures should be taken with caution.

## Data Analysis

**Voxel-Based-Morphometry**—Voxel-Based Morphometry analyses were conducted in FSL-VBM which is a part of the FSL software package (38). Brain extraction was first conducted on all participant's T1 weighted MPRAGE images using the robust version BET command, which calls the command multiple times to move the center-of-gravity closer to the true center. Afterwards, all images were checked manually to determine if the brain was adequately extracted. If the extraction was inadequate, the BET process was manually altered for each brain by changing fractional intensity threshold and/or its vertical gradient until only the whole brain was included. Next, all extracted brain images were segmented into gray matter (**GM**), white matter (**WM**) and cerebrospinal fluid (**CSF**) using FAST in FSL. This method is considered very robust and reliable (39). These images were extensively manually quality controlled to ensure no tissue type was “bleeding” into the other. This procedure has been used to prepare images for templates in previous studies (40). A pediatric study-specific template was created using the Template-O-Matic toolbox (41) toolbox in SPM. The template was made with images from an equal number of controls and girls with IBS necessary to avoid introducing systematic bias. As there were more girls with IBS than the 26 healthy controls, a random subset of 26 girls with IBS were selected. The age of every subject was entered in Template-O-Matic and was modeled as a third order regression. Afterwards, all of the GM images were non-linearly registered to the study specific template, modulated by dividing them by the Jacobian of the warp field to correct for local expansion or contraction and concatenated to create a 4D image (42). The modulated images were then smoothed with a full-width half maximum (FWHM) of 5 mm. A whole brain mask was used to investigate whole brain differences between girls with IBS and controls. This process also adjusts for total intracranial volume by modifying the volume-preserving Jacobian-modulation step such that the affine component is ignored and only the nonlinear volume changes are preserved (43). Finally, a voxel-based general linear model (**GLM**) was applied with permutation based testing at 5000 iterations was used to create clusters with the Threshold Free Cluster Enhancement (**TFCE**) method (44). All analyses were adjusted for age and site. All significance testing was conducted at  $p < .05$ , corrected for multiple comparisons using the False-Discovery Rate (**FDR**) method. Images of results were produced in Mango software (<http://ric.uthscsa.edu/mango>) and BrainNet (45).

**Resting-state fMRI Image Preprocessing**—Resting-state preprocessing was performed using Statistical Parametric Mapping 8 (SPM8) software (Wellcome Department of Cognitive Neurology, London, UK). The first three volumes were removed to allow for scanner stabilization. Images were transformed from DICOM to NIFTI format, slice-time corrected, spatially normalized to the MNI template with the MP-RAGE scan, and resampled to a voxel size of  $2 \times 2 \times 2$  mm.

**Seed-Based fMRI Seed-to-Voxel Analysis**—Preprocessed and normalized functional images were imported into the CONN-fMRI functional connectivity toolbox version 16 (46) for additional pre-processing and for seed-to-voxel connectivity analysis. Three-dimensional confounds for both white matter and CSF, obtained from segmentation procedures from preprocessing in SPM8, as well as six realignment parameters and first-order temporal derivatives of motion, were removed using regression using the CompCor procedure in CONN. White matter and CSF were confounded out using regression with 5 dimensions each. The effect of motion was regressed out using 12 dimensions. Resting-state images then were bandpass filtered between 0.01 and 0.1Hz in the CONN toolbox. The regions showing differences in the VBM analysis were used as seeds. Based on the peak voxel difference of each significantly different region in the VBM analysis, 4mm spherical seed ROIs at region were used for Seed-to-Voxel whole brain analyses. All spherical seeds were created in MarsBar (<http://marsbar.sourceforge.net>). Voxel-wise analyses were completed for each of these seeds with an initial height threshold of  $p < .001$ . Correction for multiple comparisons was done within the CONN toolbox which calculated the number of voxels in a cluster to be deemed significant at an FDR of  $p < .05$ . The final significant t-statistic connectivity maps from each seed were projected onto a normalized study-specific template in Mango software.

### Subset Analysis

**Statistical Analysis for Clinical Variables**—Independent t-tests along with Cohen's  $d$  calculations were used to determine differences and effect sizes between IBS patients and controls on clinical and quantitative sensory testing variables. Pearson's correlations were computed between GM voxel intensity for regions that were different between groups and clinical variables that were different between groups. Eigenvalues for region dyads that were significantly different between groups were extracted using SPM for each participant. Correlations between eigenvalues and clinical variables were then computed to determine associations between differences in RS-FC and clinical variables. Since pain testing was only conducted at the UCLA site, a subset analysis was performed within the UCLA sample to see how differences in GMV and FC relate to pain threshold and pain anxiety. Alpha was set to 0.05, and corrections were made using the FDR method. All statistical analyses for clinical, and quantitative sensory testing variables, as well as correlations, were conducted using R (Version 3.3.0, "Supposedly Educational") and RStudio (Version 0.99.447).



## Results

### Clinical Data

The mean age of the UCLA IBS and HC samples did not differ ( $14.53 \pm 2.91$  vs  $12.05 \pm 3.13$ ). The mean age of the Baylor IBS and HC samples also were similar ( $9.76 \pm 1.26$  vs  $9.16 \pm 1.11$ ).

### Clinical Data – Combined Sample

Independent sample t-tests revealed that trait anxiety measured via the STAI was significantly higher in girls with IBS in the UCLA sample ( $t_{(21,17)} = 2.15, p = .043, d = 0.87$ ) and Baylor sample ( $t_{(14,87)} = 3.16, p = 0.007, d = 1.48$ ) compared to HC. There was no difference in trait anxiety between the UCLA and Baylor samples ( $t_{(22,25)} = -1.32, p = .20, d = 0.57$ ). See Table 1.

### Voxel-Based Morphometry – Combined Sample

IBS patients exhibited lower GMV in limbic, paralimbic, prefrontal, and temporal regions. See Table 2 for a summary of regions showing significant differences and Figure 1 for a visual depiction.

### Resting State Functional Connectivity (Seed-to-Voxel) – Combined Sample

Girls with IBS exhibited lower connectivity between the bilateral anterior mid-cingulate cortex (aMCC) and right precuneus. They also exhibited greater connectivity between the right CaN and left precentral gyrus compared to HC. Significant differences between groups from the seed-to-voxel analysis is displayed in Table 3, Figure 2 and Figure 3.

## Subset Results (UCLA participants only)

### Laboratory pain testing

There was no significant difference between IBS (mean = 39.22, SD = 18.98) and HC (mean = 31.00, SD = 13.21) in pain specific anxiety measured using the CPASS ( $t_{(17,15)} = 1.22, p = .24, d = .51$ ). There was no difference between IBS patients (mean = 1.82, SD = 2.23) and HC (mean = 2.21, SD = 2.58) in current anticipatory anxiety prior to the laboratory pain tasks ( $t_{(19,84)} = -0.58, p = 0.57, d = -.24$ ). Finally, there was a significant difference between HC (mean = 0.86, SD = 1.35) and IBS (mean = 2.82, SD = 2.64) in current pain rating ( $t_{(14,08)} = 2.24, p = .041, d = 0.97$ ).

Independent t-tests revealed differences between HC (mean = 43.78°, SD = 4.72) and girls with IBS (mean = 46.41°, SD = 3.34), with IBS patients showing higher, but insignificant (perhaps due to low power), baseline pain threshold ( $t_{(22,81)} = 1.63, p = 0.12, d = 0.63$ ).

### Voxel-based Morphometry

To investigate how discrepancies in GMV and FC relate to pain threshold, the UCLA sample was run through the VBM and CONN procedures separately. In this smaller sample, significant differences remained with IBS patients exhibiting lower GMV in the thalamus bilaterally, left CaN and left nucleus accumbens. See Table 4 for a summary. GM intensity

values for each participant were then extracted and correlations were run against variables that showed significant differences between groups. Lower GMV in the thalamus bilaterally (Left:  $r_{(22)} = -.62$ ,  $p = .001$ ,  $p_{(FDR)} = .008$ , Right:  $r_{(22)} = -.51$ ,  $p = 0.01$ ,  $p_{(FDR)} = 0.04$ ) was associated with increased pain thresholds. See Figure 4. No correlations were observed with any other clinical measures.

### Resting State Functional Connectivity (Seed-to-Voxel)

Based on the results of the UCLA subsample VBM analysis, regions showing differences were used as seeds in a seed-voxel RS-FC analysis. IBS patients exhibited lower connectivity from the left CaN to the left middle frontal gyrus (dlPFC), compared to connectivity in those regions in HC (Ke (cluster size in voxels) = 53,  $\beta = -0.15$ ,  $t = -6.45$ ,  $p_{(FDR)} < 0.001$ , MNI Coordinates: X = -42, Y = 26, Z = 34). IBS patients also exhibited greater connectivity between the left thalamus and left posterior parietal cortex, a key node of the default-mode network (Ke = 48,  $\beta = 0.14$ ,  $t = 6.66$ ,  $p_{(FDR)} < 0.001$ , MNI Coordinates: X = -40, Y = -52, Z = 36). A visual depiction can be seen in Figure 5. No connectivity correlations were observed with clinical or behavioral variables.

## Discussion

The alterations in brain structure described here in girls with IBS show striking similarities to prior studies in adults with IBS (23). Girls with IBS have reduced gray matter volume in key brain regions involved in sensory, salience and default-mode networks. Previous studies investigating children with chronic pain have also observed decreases in GMV in regions such as the caudate nucleus, nucleus accumbens, putamen, dlPFC, aMCC, and PCC (32,47). Unlike adults with IBS and other pain disorders, in whom increases in volume or cortical thickness have been noted in the somatosensory cortex, no gray matter increases were identified in this study (23,48,49). Girls with IBS also demonstrated lower resting state neural connectivity between the salience (aMCC) and default-mode (precuneus) networks, and between the sensorimotor (caudate nucleus) and central-executive network (dlPFC). Girls with IBS exhibited greater neural connectivity within the sensorimotor network (caudate nucleus and motor cortex). This suggests an alteration in the intraregional brain communication required for normal sensory processing. Furthermore, thalamus brain volume in girls with IBS was associated with pain thresholds, suggesting that this impaired communication in the sensory relay center may be related to the interpretation of pain stimuli in IBS.

### Structural and functional brain alterations in girls with IBS

Our analyses found that GMV differences were prominent in regions involved in determining salience from external and internal stimuli and regulating appropriate physiological responses, (50–52) such as the aMCC, dlPFC, and ventral striatum. IBS patients also had lower GMV in the sensory regions including the thalamus, along with the dlPFC. Furthermore, patients with IBS exhibited lower RS-FC between aMCC and the precuneus, suggesting that the salience network has less integration with the default-mode network in girls with IBS. The aMCC within the salience network is involved in modulating responses in the sensorimotor and association cortices, and is considered to generate the



hypervigilance to body sensations found in IBS (53–55). In neuroimaging of a visceral pain task in adolescents with IBS, the IBS group was noted to have increased connectivity of the salience network with executive control network but not sensory regions, suggesting that even in the setting of active visceral stimulation, the most prominent abnormality in IBS is in determining signal salience (54). Girls with IBS also exhibited lower RS-FC between the caudate nucleus and dlPFC, suggesting lower integration between the sensorimotor and central-executive networks, or less inhibitory control over sensory stimuli. The greater connectivity observed between the caudate and precentral gyrus and between the bilateral thalamus and PPC suggest greater communication within the sensorimotor network, and to a region in the default-mode network responsible for self-centered mental imagery and self-referential processing (56). We hypothesize that a combination of these processes could increase painful communication and interoceptive rumination in girls with IBS.

### **Pain sensitivity in IBS**

While pain is the cardinal symptom distinguishing IBS from other functional disorders involving bowel habits, the experimental data on pain sensitivity is highly variable and the degree of sensitivity often does not correlate well with clinical symptoms. Visceral sensitivity, measured by balloon distension in the gut, was initially thought to be a biomarker of the disorder in adults, however it is now well established that only a portion of IBS patients are hypersensitive, some are normosensitive and some are even hyposensitive (57). Somatic sensitivity in adults and children with IBS has been similarly variable (13,15–17). In this study, girls with IBS tended toward higher somatic pain thresholds compared to HC, as has been observed in children and adult females with IBS (15,20). More importantly, the girls with IBS show alterations in the brain structure in regions that have a key role in pain processing and rumination (58), such as the thalamus, basal ganglia, precuneus and aMCC, as well as lower functional connectivity of pain regions with the dlPFC, suggesting that patients with IBS may have a suppressed endogenous analgesic (pain modulation) system (58,59). Correlations between experimental pain sensitivity and brain structure (thalamic GMV) in girls with IBS also differed.

### **Limitations**

This study is limited in that it views only a cross-sectional state in children who have already developed IBS symptoms. This makes it impossible to determine whether the brain differences identified are predisposing factors to developing IBS or the result of the symptoms. While longitudinal and symptom intervention studies are required to better answer this question, the presence of these findings in this young group of children is suggestive that the brain in IBS is developing its salience and sensory circuitry differently at an early age. This study is also limited by the exclusion of boys. With the known sex differences in pain processing and brain development, these results cannot be extrapolated to male IBS patients. The size of the current sample must also be taken into consideration, with larger studies needed for reliable replication.

### **Conclusions**

As has been shown in adults with IBS, girls with IBS have significant brain structural and functional differences compared to healthy peers. Disruption of the salience network and its

communication with other brain regions should be a target of additional studies across the lifespan. Insight into the clinical relevance of these differences will be elucidated via studies incorporating interventions aimed at brain-gut neural signaling to capture those brain changes most associated with symptom improvement. Children, with rapidly developing brains and a risk for lifelong vulnerability to chronic pain (60,61), should be a focus of such studies.

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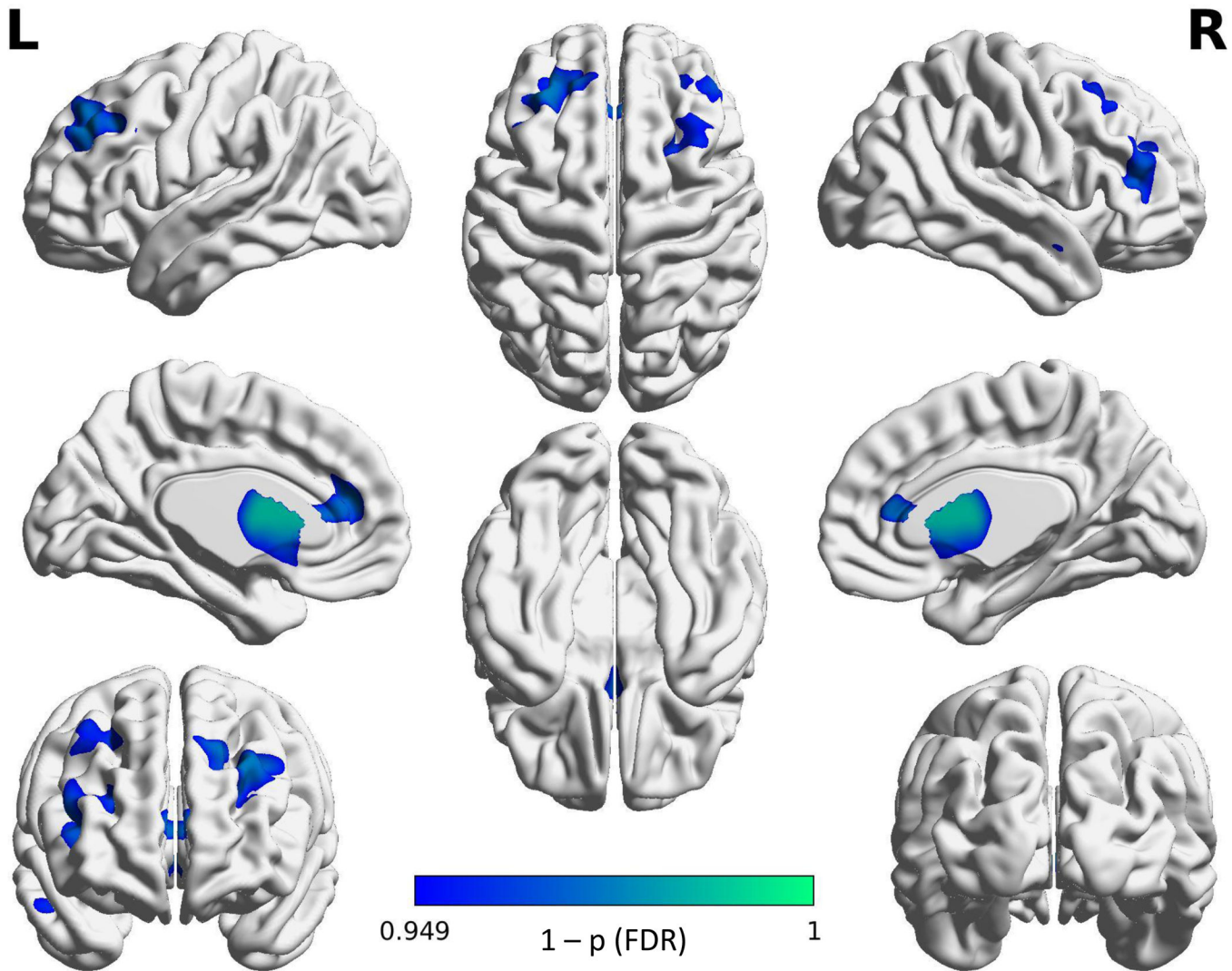
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**Figure 1: GM differences between IBS and HC in the Combined Sample**

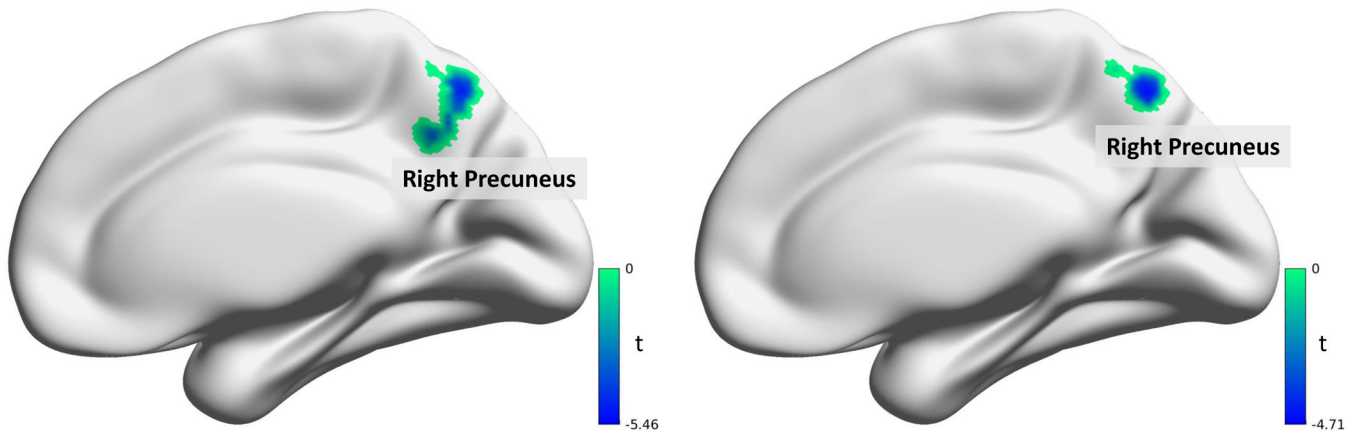
IBS < HC contrast shows regions in IBS that were significantly lower in GMV than in the HC.

Projected color map displays FDR corrected “1-p” values.

The combined sample shows IBS patients having lower gray matter volume in multiple regions of the brain including the dorsolateral prefrontal cortex (dlPFC), anterior mid-cingulate cortex (aMCC), thalamus and basal ganglia. Color map represents “1-p” values with lighter values being closer to 1. All results shown are significant at  $p_{(FDR)} < .05$ .

Seed: Left Mid-Anterior Cingulate Cortex (aMCC)

Seed: Right Mid-Anterior Cingulate Cortex (aMCC)

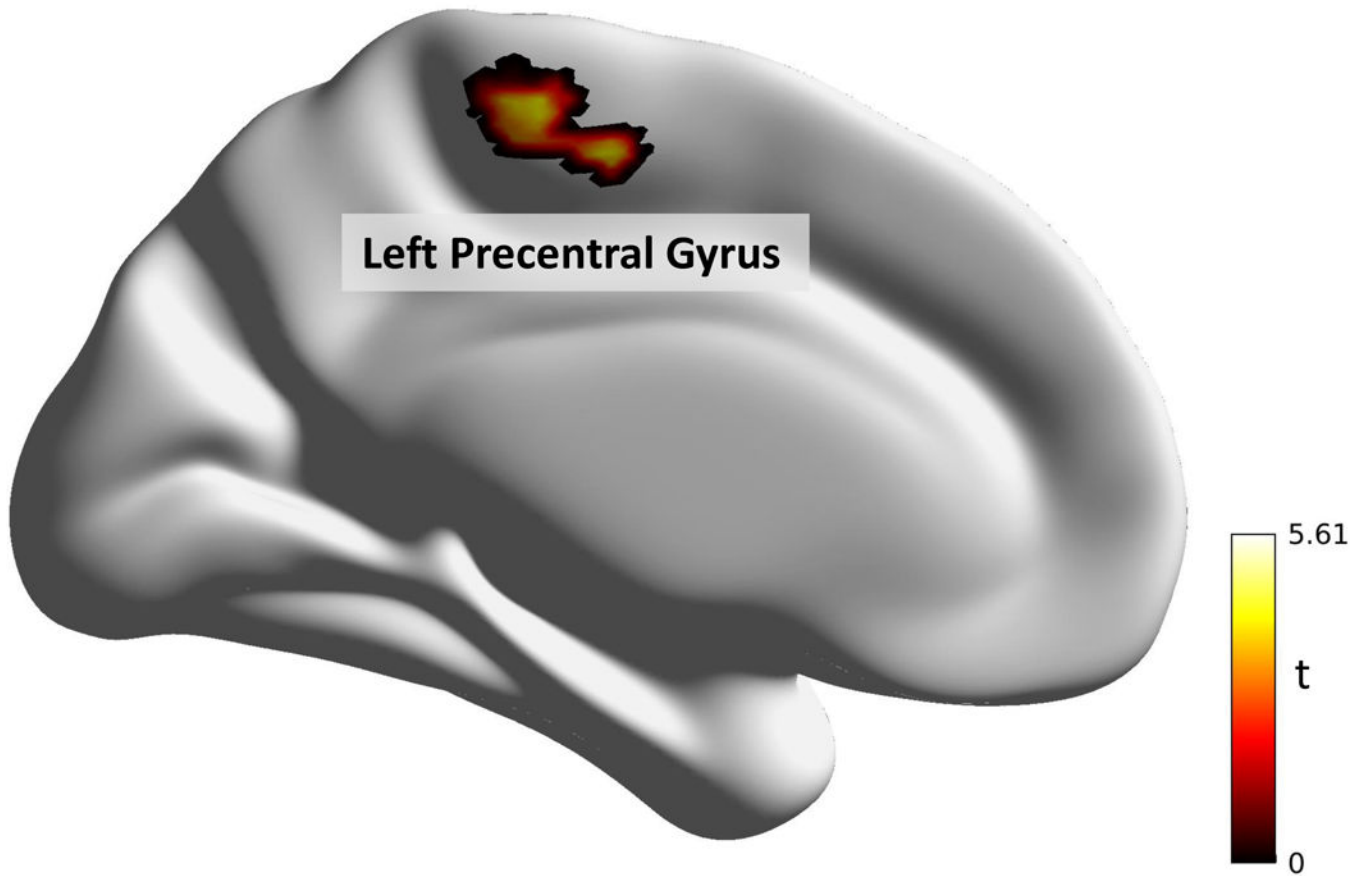


**Figure 2: RS-FC differences between IBS and HC in the Combined Sample**

The combined sample shows girls with IBS having lower connectivity from the bilateral aMCC to the right precuneus. All results shown are significant at  $p_{(FDR)} < .05$

**Abbreviations:** *t*: t-value.

## Seed: Right Caudate Nucleus

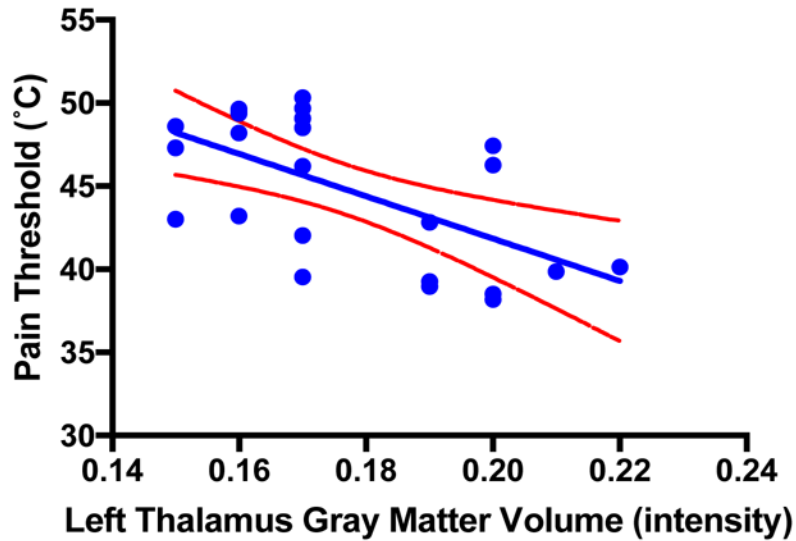


### Figure 3: RS-FC differences between IBS and HC in the Combined Sample

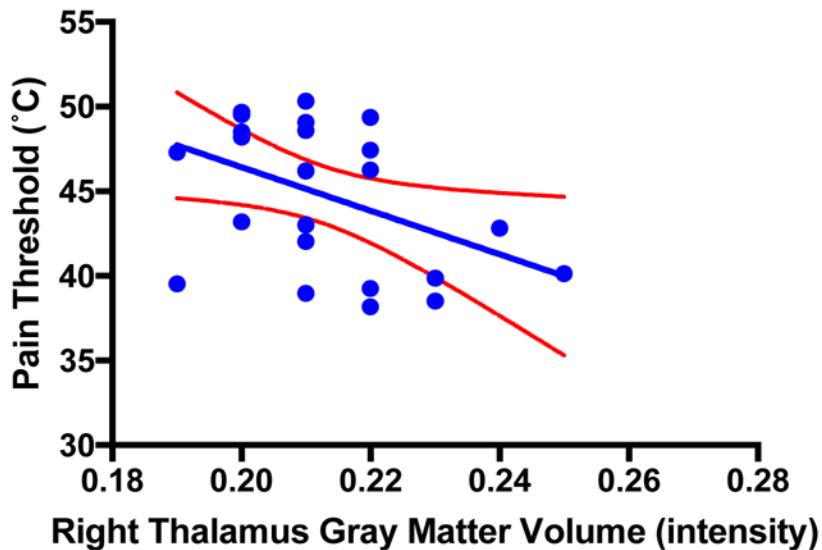
The combined sample shows girls with IBS having greater connectivity from the right caudate nucleus to the left precentral gyrus. All results shown are significant at  $p_{(FDR)} < .05$ .

**Abbreviations:** *t*: t-value.

### Lower Left Thalamus GM is associated with Greater Pain Thresholds

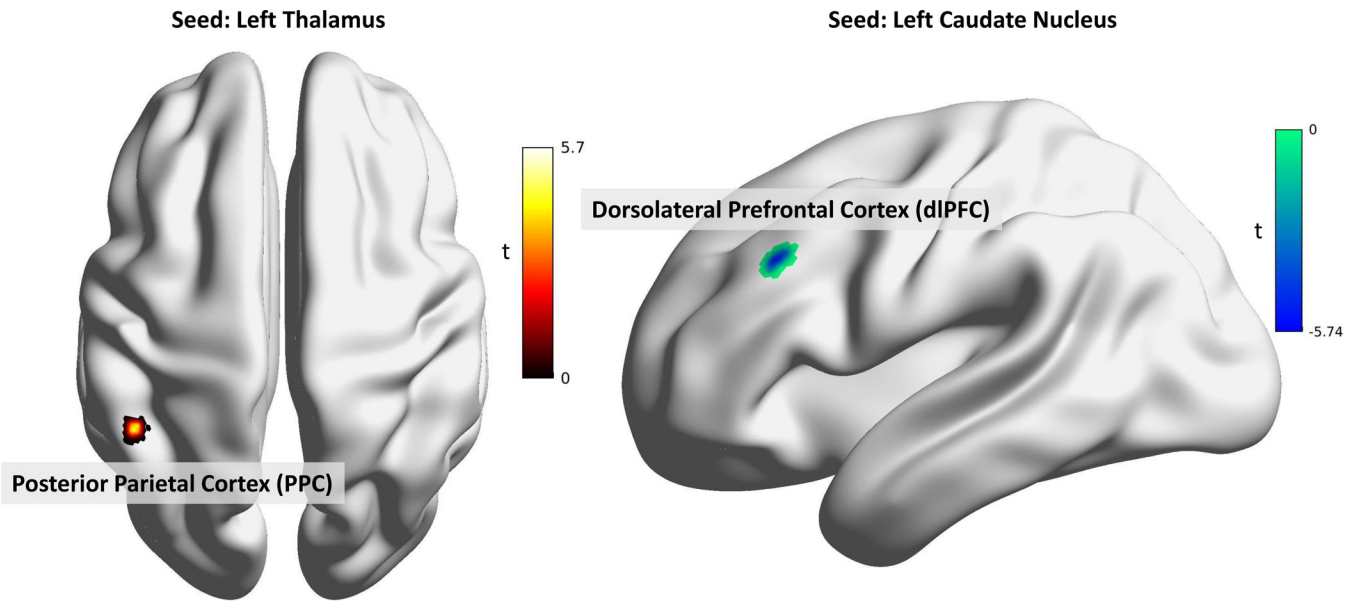


### Lower Right Thalamus GM is associated with Greater Pain Thresholds



**Figure 4:**

UCLA Sample – Lower GMV in the Thalamus bilaterally (Left:  $r = -.62$ ,  $p = .001$ ,  $p_{(FDR)} = 0.008$ ; Right:  $r = -.51$ ,  $p = .01$ ,  $p_{(FDR)} = 0.04$ ) is associated with increased pain thresholds. Blue lines represent line of best fit and red dotted lines represent 95% confidence intervals.

**Figure 5:**

In the UCLA Sample, patients with IBS exhibited greater connectivity between the left thalamus and left posterior parietal cortex and lower connectivity between the left caudate nucleus and the left middle frontal gyrus (dlPFC) compared to HC. All results shown are significant at  $p_{(FDR)} < .05$ .

**Abbreviations:** *t*: t-value.

**Table 1:**

Clinical variables of both (UCLA and Baylor) study sites.

UCLA Sample		IBS (N = 10)		HC (N = 14)				
Variable	Mean	SD	Mean	SD	<i>t</i>	<i>p</i>	<i>d</i>	
Age	14.54	2.91	12.05	3.13	2.05	0.06	0.82	
State-Anxiety (STAI)	31.45	4.70	32.57	5.26	-0.56	0.58	-0.22	
Trait-Anxiety (STAI)	43.10	8.38	35.86	8.04	2.15	0.043	0.87	
Child Pain Anxiety Symptoms Scale (CPASS)	39.22	18.98	31.00	13.21	1.22	0.24	0.51	
Current Pain (NRS)	2.82	2.64	0.86	1.35	2.24	0.041	0.97	
Anxiety Prior to Pain Task (NRS)	1.82	2.23	2.21	2.58	-0.58	0.57	-0.24	
Pain Threshold (°C)	46.41	3.34	43.78	4.72	1.63	0.12	0.63	
Baylor Sample		IBS (N = 22)		HC (N = 12)				
Variable	Mean	SD	Mean	SD	<i>t</i>	<i>p</i>	<i>d</i>	
Age	9.76	1.26	9.16	1.11	1.40	0.17	0.49	
Trait-Anxiety (STAI)	38.50	7.29	28.71	5.47	3.16	0.007	1.48	
Number of pain episodes	10.81	8.54	0.25	0.45	5.65	1.5e-5	1.54	

**Abbreviations:** STAI: State-Trait Anxiety Inventory; NRS (Numeric Rating Scale), °C (degrees in Celsius).



**Table 2:**

Clusters and regions defined by their MNI coordinates that showed significant differences between IBS patients and HC in the combined sample.

Region	Voxels	Hemisphere	<i>t</i>	<i>p</i> (FWE)	MNI Coordinates		
					X	Y	Z
<b>Cluster 1 (IBS &lt; HC)</b>							
Caudate Nucleus	5100	Left	3.33	0.027	-6	14	6
Caudate Nucleus		Right	4.04	0.014	8	2	12
Nucleus Accumbens (Nacc)		Left	3.16	0.031	-6	14	-2
Nucleus Accumbens (Nacc)		Right	2.89	0.041	6	12	-4
Thalamus		Left	3.57	0.022	-2	-4	8
Thalamus		Right	3.87	0.017	4	-2	8
Anterior Mid-Cingulate Cortex (aMCC)		Left	3.54	0.029	0	36	12
Anterior Mid-Cingulate Cortex (aMCC)		Right	3.54	0.029	4	36	12
Dorsolateral Prefrontal Cortex (dlPFC)		Left	3.76	0.026	-30	40	38
<b>Cluster 2 (IBS &lt; HC)</b>							
Dorsolateral Prefrontal Cortex (dlPFC)	1714	Right	4.28	0.032	42	38	10
Dorsolateral Prefrontal Cortex (dlPFC)		Right	3.51	0.040	24	14	50
<b>Cluster 3 (IBS &lt; HC)</b>							
Superior Temporal Gyrus, Anterior Division	139	Right	4.19	0.042	54	0	-20

Combined sample VBM results show patients with IBS exhibit lower gray matter volume throughout the brain including the dorsolateral prefrontal cortex (dlPFC), anterior cingulate cortex (ACC), thalamus, basal ganglia regions, and superior temporal gyrus.

**Table 3:**

Combined Sample Resting-State Functional Connectivity Results.

						MNI Coordinates		
Seed	Region	Ke	beta	<i>t</i>	<i>p</i> <sub>(FDR)</sub>	X	Y	Z
<b>Contrast: IBS &lt; HC</b>								
L aMCC	R Precuneus	127	.13	6.34	.004	6	-64	52
R aMCC	R Precuneus	87	.13	4.92	.04	6	-64	52
<b>Contrast: IBS &gt; HC</b>								
R CaN	L Precentral Gyrus	105	.11	5.93	.008	-10	-16	56

**Abbreviations:** L: left, R: right, aMCC: anterior mid-cingulate cortex, CaN: Caudate Nucleus, Ke: number of voxels, beta: beta-value, *t*: t-value, *p*(FDR): p-value corrected for false-discovery rate

Seeds were chosen based on significant group differences in the VBM analyses. Only dyads that showed significant differences are shown. Locations of clusters are expressed in MNI-space.

**Table 4:**

Regions defined by their MNI coordinates that showed significant GM differences in the UCLA sample between IBS patients and HC.

Region	Voxels	<i>t</i>	<i>p</i> (FDR)	MNI Coordinates		
				X	Y	Z
<i>CLUSTER (IBS &lt; HC)</i>	953	3.45	0.001	-6	16	-14
L Caudate Nucleus		5.01	0.002	-10	14	0
L Nacc		5.15	0.002	-10	-16	-8
R Thalamus		4.56	0.002	10	-12	18
L Thalamus	4	3.82	0.002	-16	-28	14

Sample show female adolescents with IBS exhibit lower GMV in the bilateral thalamus and left caudate nucleus and left nucleus accumbens.

**Abbreviations:** Nacc: nucleus accumbens. L: left, R: right, *t*: t-value, *p*(FDR): corrected p-value using the false discovery rate.