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Altered viscerotopic cortical innervation in patients with irritable bowel syndrome

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Abstract

Background—Studies have demonstrated the existence of regional gray matter and white matter (WM) alterations in the brains of patients with irritable bowel syndrome (IBS), but the extent to which altered anatomical connectivity between brain regions is altered in IBS remains incompletely understood.

Methods—In this study, magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) were used to identify significant brain connectivity differences between IBS patients and healthy control (HC) subjects. Based on MRI and DTI volumes acquired from 66 IBS patients and 23 HC subjects, multivariate regression was used to investigate whether subject age, sex, cortical thickness or the mean fractional anisotropy (FA) of WM connections innervating each location on the cortex could predict IBS diagnosis.

Key results—HC and IBS subjects were found to differ significantly within both left and right viscerotopic portions of the primary somatosensory cortex (S1), with the mean FA of WM bundles innervating S1 being the predictor variable responsible for these significant differences.

Conclusions and inferences—These preliminary findings illustrate how a chronic visceral pain syndrome and brain structure are related in the cohort examined, and because of their indication that IBS diagnosis is associated with anatomic neuropathology of potential neurological relevance in this patient sample.

Keywords

connectomics; diffusion tensor imaging; irritable bowel syndrome; magnetic resonance imaging; neuroimaging; somatosensory cortex

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Individual contributions statement

AI and JDVH designed the research study; AI and CMT performed the research; AI, JSL and EAM contributed essential tools; AI, CMT and JDVH analyzed the data; AI wrote the paper.

Introduction

Functional and structural alterations in the complex bidirectional interactions between the gastrointestinal (GI) tract and the human brain have been reported in chronic abdominal pain syndromes (1). Studies have demonstrated the existence of regional gray matter (GM) and white matter (WM) alterations in the brains of patients with irritable bowel syndrome (IBS) (2-4), but the extent to which altered anatomical connectivity between brain regions is altered in IBS remains incompletely understood. Previous studies have examined differences in cortical thickness between IBS patients and healthy control (HC) subjects, as well as sex-related differences in brain structure between the two populations (5). In addition, significant differences in the properties of certain WM connections have been found between IBS patients and HC subjects (4). Nevertheless, no study has yet explored whether and how various cortical locations are innervated differently in IBS patients compared to healthy controls. The aim of this research is to use multimodal neuroimaging in conjunction with multivariate statistical analysis in order to identify how cortical regions are innervated differently in IBS patients compared to HC subjects. The hypothesis of the study is that IBS patients and HC subjects exhibit statistically significant differences with regard to the WM connections which innervate each cortical location. In addition to presenting the results of testing this hypothesis, we discuss the potential implications of this finding in the context of previous studies.

Materials and methods

Participants

Both HC and IBS participants provided their informed written consent as required by the Declaration of Helsinki, U.S. 45 CFR 46. All individuals were enrolled at the UCLA Center for Neurobiology of Stress between 2007-13. The study was conducted with approval from the UCLA Institutional Review Board and included $N = 89$ subjects (23 HC males, 33 HC females, 19 IBS males, 14 IBS females; average age over groups: 38.6 ± 11.1 years; average HC age: 38.6 ± 11.51 years; average IBS patient age: 38.7 ± 10.4 years). IBS subjects met Rome II or III symptom criteria for IBS as assessed by a gastroenterologist or nurse practitioner. Bowel habit (BH) was quantified using the following scale: 1 = constipation; 2 = diarrhea; 3 = alternating; 4 = normal; 5 = unspecified; 6 = mixed. The Hospital Anxiety and Depression (HAD) and State Trait Anxiety Inventory (STAI) tests were administered to each subject. The Bowel Symptom Questionnaire (BSQ) was administered to the IBS patients, and the following scores with values between 0 and 20 were recorded: overall symptoms in the past week, abdominal pain in the past week, bloating in the past week, usual symptom severity (1 = none, 2 = mild, 3 = moderate, 4 = severe, 5 = very severe), age of IBS onset, and IBS duration in years. The Patient Health Questionnaire (PHQ) was administered to all subjects, including the PHQ-15, PHQ-13 (no IBS-related questions), and the PHQ-12 (no GI-related questions).

Neuroimaging

A Trio Tim scanner (Siemens Corp., Erlangen, Germany) was used to acquire T_1-weighted MRI and DTI volumes at 3 T from each participant. For MRI, an MP-RAGE sequence was

used with repetition time (*TR*) = 2 s, echo time (*TE*) = 28 ms and 2 mm slice thickness. The DTI sequence had *TR* = 9.4 s, *TE* = 88 ms, 2 mm slice thickness, 68 gradient directions, matrix size of 128 × 128, field of view (FOV) of 256 mm, and no inter-slice gap. In each subject, DTI and MRI volumes were subjected to affine co-registration, and each set of DTI fiber tracts were reconstructed via deterministic tractography. Individual fibers less than 15 mm in length were discarded. The cortical surface of the brain was reconstructed as a triangular tessellation with ~300,000 vertices (average inter-vertex distance: ~1 mm) to produce an anatomically faithful, smooth representation of the GM/WM interface (6).

Image processing

Cortical inter-connectivity and cortical thickness were calculated as detailed elsewhere (7). Briefly, at each vertex v_i of the tessellation, cortical thickness was measured as the distance between the GM/WM boundary and the cortical surface. The mean FA of each WM fiber bundle was calculated as the average FA over all DTI voxels traversed by the fiber bundle along its path. DTI and MRI volumes were first co-registered using affine registration. Eddy current correction was then applied to each DTI volume, which was subsequently processed using TrackVis (http://trackvis.org) to reconstruct fiber tracts using deterministic tractography. Mean FA maps for connections innervating each point on the cortex were smoothed across using a circularly symmetric Gaussian kernel with a full width at half maximum of 5 mm and averaged across subjects using a non-rigid, high-dimensional spherical averaging method to align cortical folding patterns (6).

Brain connectivity calculation

After cortical parcellation and WM tractography, the connectivity matrix of each subject was calculated. Let v_i and v_j be cortical mesh vertices linked by some WM connection c_{ij}. For each such connection, the three-dimensional coordinates associated with the extremities of c_{ij} (i.e. with v_i and v_j) were identified. The corresponding entry indexed by *i* and *j* in the matrix $\boldsymbol{C}$ of the subject in question was updated to reflect the presence of a connection between v_i and v_j. This process was repeated for each connection. The mean FA of c_{ij} was computed as the average of FA values over all DTI voxels traversed by c_{ij} from one end of the connection to the other end. In a similar way, at each vertex v_i on the cortical mesh**,** the mean FA of connections linking v_i to the rest of the brain was computed. The CD at each vertex was calculated as the sum of all fibers linking it to the rest of the brain, divided by the area of the vertex neighborhood and by the total number of brain connections. Here, the neighborhood of vertex v_i denotes the portion of the mesh surface containing points which are closest to v_i.

Statistical analysis

For each point on the cortex, multivariate regression was used to investigate whether any of the independent feature variables (subject age, sex, cortical thickness and the mean FA of connections innervating the cortex at the location in question) could predict the response variable (IBS diagnosis). Because the psychological profile variables were found to be (A) substantially inter-correlated (r = 0.42 to r = 0.97) and (B) additionally correlated with age (r = 0.38), and additionally because (C) age was already included in the design matrix,

psychological profile variables were not included in the analysis in order to avoid violating the assumptions of the general linear model, which requires predictor variables to be statistically independent (8, 9). If the design matrix includes substantially correlated predictor variables, the column vectors of the design matrix are no longer orthogonal, i.e. they are linear combinations of each other. In such a situation, the design matrix is rank-deficient and it is not possible to determine which of the inter-correlated variables actually has a predictive effect upon the response variable (10). This situation is known as multicolinearity and should be avoided in multivariate statistical analysis because it leads to ambiguity in interpreting the results of the analysis (11, 12).

The omnibus null hypothesis that none of the independent variables predicts IBS diagnosis was tested using a standard multivariate regression approach (8). To test whether any of the independent variables can *individually* predict IBS diagnosis, a 'leave-one-out' reduced-model regression was implemented where the null hypothesis was that the removed variable does not contribute to the regression above and beyond all other variables. Statistical significance was tested at the $\alpha = 0.05$ level subject to the false discovery rate (FDR) correction for multiple comparisons. A power analysis was also implemented using a standard approach (13) to investigate whether our sample size was large enough to detect an effect size η^2 equal to 0.1 at significance levels α of 0.05 and 0.01.

In addition to the regression model described above, the significance of the univariate correlation between mean FA, on the one hand, and each of (A) IBS duration and (B) IBS severity was also tested. With *r* being the Pearson product moment correlation coefficient, the statistic $t = r / \sqrt{(1 - r^2) / (N - 2)}$ has Student's *t* distribution with $N - 2$ d. f. The null hypothesis that no significant correlation exists between each of the pairs of quantities being considered was tested at $\alpha = 0.05$ subject to the false discovery rate (FDR) correction for multiple comparisons.

Results

Demographic information for the IBS and HC samples is reported in Table 1, which indicates that the HC and IBS groups differ significantly as quantified using the selected metrics of BH, BMI, depression, anxiety and physical health. For the neuroimaging portion of the study, the power analysis indicated that, for α values of 0.05 and 0.01, the statistical power π of the study was equal to 0.954 and 0.951, respectively, for an effect size $\eta^2 = 0.1$. The power analysis also revealed that, to achieve $\pi = 0.8$ with $\alpha = 0.01$ and $\eta^2 = 0.1$, a sample size of 48 subjects would have sufficed. The omnibus statistical analysis showed that the multivariate feature vectors of HC and IBS subjects differ significantly within both left and right viscerotopic portions of the primary somatosensory cortex (S1). Specifically, Figure 1A displays the results of the multivariate regression analysis to determine the extent to which subject age, cortical thickness at the location of cortical mesh vertex v_i, and the mean FA of connections innervating the cortex at vertex v_i can predict IBS diagnosis. For each cortical location v_i, the *F* statistic with 4 and 84 degrees of freedom (i.e. $F_{4,84}$) is displayed for the omnibus test of the null hypothesis that none of the three independent variables predicts IBS diagnosis. Figure 1B displays the results of testing the null hypothesis

that the mean FA of connections innervating the cortex at v_i does not contribute to the regression model above and beyond all other predictor variables. The test statistic is Student's *t* with 84 d. f. (i.e. t_{84}) and its sign is positive, indicating significantly higher mean FA in IBS patients compared to HC subjects. In both (A) and (B), the displayed values of the test statistic are thresholded for significance using the Benjamini-Hochberg False Discovery Rate (FDR) method with $\alpha < 0.05$ and cortical locations for which the null hypothesis is not rejected are drawn in white. Arrows indicate cortical locations for which the null hypothesis is not accepted.

In the right hemisphere, the cortical location exhibiting the maximum statistical difference between the two groups was identified at Talairach coordinates (−60.91 mm, −12.85 mm, 21.17 mm); in the left hemisphere, the corresponding location was (61.99 mm, −8.29 mm, 23.74 mm). Thus, the identified statistical differences are unambiguously localized in S1. The *post-hoc* reduced-model tests further reveal that the mean FA of WM bundles innervating S1 is the *only* predictor variable responsible for these significant differences between the two populations, with significantly *higher* mean FA in the IBS group (Figure 1B). No statistically significant sex-related or cortical thickness-related differences were found between the two groups, and no other cortical areas were found to exhibit significant differences between groups. In addition, no significant correlations were found between either IBS duration or severity, on the one hand, and mean FA, on the other hand, at a significance level of $\alpha = 0.05$, subject to the FDR correction for multiple comparisons. For illustrative purposes, a visual depiction of the WM connections innervating S1 in a representative patient is shown in Figure 2. The WM connections between the postcentral gyri and the corticospinal tracts are drawn in bright green and highlighted by green arrows; for other connections, tract color indicates fiber direction (blue: tracts oriented along the antero-posterior axis; orange: tracts oriented along the inferior-superior axis). The WM of the left hemisphere is depicted in a translucent shade of white to provide a spatial reference context. The WM of the right hemisphere is not shown in order to allow the reader to visualize the WM connections more easily.

Discussion

The main finding of this study is that, interestingly, IBS patients exhibit significant abnormality in the structural connectivity properties of the viscerotopic portion of S1, which is functionally involved in processing afferent signals from the GI tract. In humans, the inferior extremity of the postcentral gyrus is located on the lateral aspect of the parietal lobe just above the Sylvian fissure and has long been acknowledged as the portion of S1 responsible for processing sensory perceptions and pain from the viscera, including the esophagus, stomach, duodenum and sigmoid colon (14, 15). In this context, the existence of significant differences between IBS patients and HC subjects in the mean FA of WM connections innervating the viscerotopic portion of S1 may be indicative of differences in WM fiber diameter and/or myelination between the two populations.

Functional studies involving functional MRI (fMRI), positron emission tomography (PET) and electroencephalography (EEG) have found increased viscerotopic brain activation relative to baseline activity in response to visceral stimulation (16, 17). In one experiment

involving barostat-controlled visceral stimulation (18), stimulus-related fMRI activity volume was significantly higher in IBS patients compared to HC subjects, and areas of involvement included sensorimotor cortex, as in our study. Similarly, a detailed and systematic literature review of brain activation patterns in response to visceral stimulation found that the sensorimotor network—including the primary somatosensory cortex—is involved in brain responses to visceral pain, according a number of studies which used functional, metabolic and electrophysiological measurement techniques [see (19, 20) and references therein]. Nevertheless, these studies involved small sample sizes of ~10 IBS patients or even fewer, whereas our present findings have the advantage of being based on a much larger sample (N = 89). Furthermore, these studies investigated *functional* activation differences between IBS patients and HC subjects, whereas ours is a study of *structural* differences. For example, functional brain activation in response to visceral pain has been variously described as being localized in the inferior part of S1 or in S2, which are two very closely related regions (20). In our study, however, the structural differences of interest were identified in S1 alone. This may suggest that functional activation patterns specific to IBS patients may partially differ from structural differences, and that additional studies may be required to understand the differences and commonalities between the two.

In one previous studies pertaining to brain connectivity differences in IBS patients, sex-related differences were found at specific locations within the WM by Ellingson et al. (4); by contrast, in our study, such differences were not found at a statistically significant level. This may indicate that, whereas the properties of some WM connections may differ between sexes in IBS patients (4), the mean FA over all connections innervating each cortical region is different in IBS versus HCs only in the viscerotopic portion of S1 (as found in the present study). This subtle yet important distinction highlights the complexity of structural brain differences between males and females in IBS.

The preliminary findings of the present study may provide a unique example of how IBS and brain structure are related in the sample examined, and are interesting because of their indication that IBS diagnosis in these patients is associated with structural brain differences of potential interest to clinicians. Indeed, our results indicate that IBS may involve altered viscerotopic connectivity along corticospinal pathways innervating S1. This highlights prominently the possible relationship between GI disease in general—and IBS in particular—and the viscerotopic circuitry of the cerebral cortex.

For at least several reasons, the significance of the present study should be viewed as primarily methodological. Firstly, future studies in larger samples will likely need to address the possible role of sex-related effects upon brain structure in IBS (2-4). Additionally, such studies should include positive control subjects to increase statistical specificity (the absence of such subjects here should be acknowledged as a limitation). Although the present study includes a total of 89 subjects, inclusion of a larger sample size would additionally lead to increased statistical power in detecting differences of interest between the two populations. Finally, further study is needed to investigate whether the altered structural connectivity reported here is (A) a cause of IBS, (B) a consequence of this condition, (C) a risk factor for it, or rather (D) due to a reciprocally modulatory relationship between IBS symptoms and the viscerotopic circuitry alterations described.

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Key messages

- White matter (WM) connections innervating the viscerotopic portion of the primary somatosensory cortex (S1) are significantly different in irritable bowel syndrome (IBS) patients compared to healthy control (HC) subjects, indicating that IBS diagnosis is associated with anatomic neuropathology of potential neurological relevance;
- Magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) were employed in conjunction with multivariate statistical analysis to identify significant brain connectivity differences between IBS patients and HC subjects;
- Based on MRI and DTI volumes acquired from 66 IBS patients and 23 HC subjects, multivariate regression was used to investigate whether subject age, cortical thickness and the mean fractional anisotropy (FA) of WM connections innervating each location on the cortex could predict IBS diagnosis;
- HC and IBS subjects were found to differ significantly within both left and right viscerotopic portions of S1, with the mean FA of WM bundles innervating S1 being the predictor variable responsible for these significant differences between the two populations.

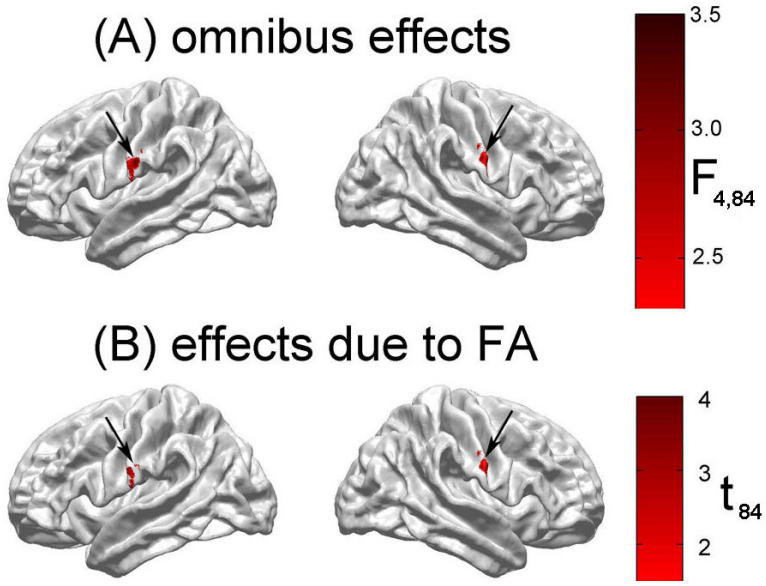

Figure 1.

(A) Cortical locations innervated by WM circuitry which differs significantly between IBS patients and HC subjects. (B) Locations where the mean FA of connections innervating the cortex is statistically significantly different in IBS patients compared to HC subjects (see *Results* section for details).

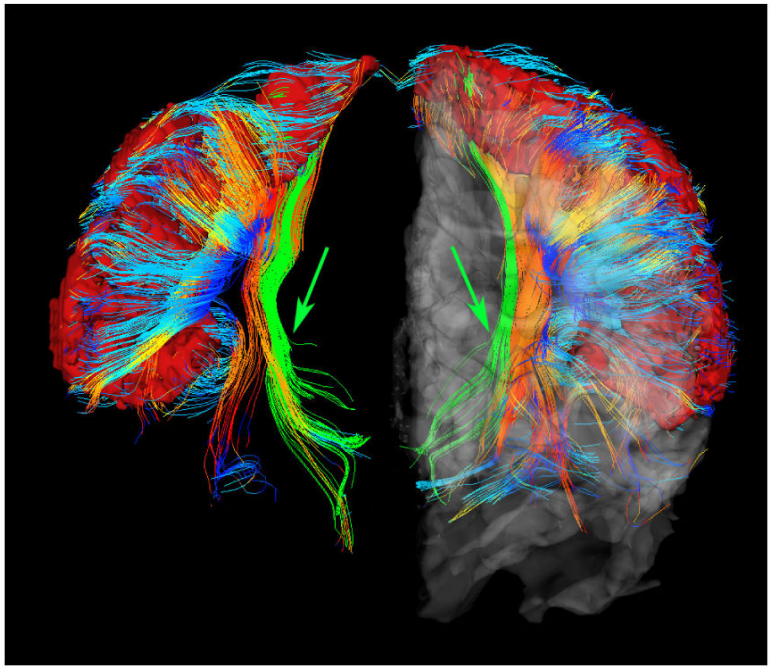

Figure 2.
Visual representation of WM connections innervating S1 in a representative study patient. Depicted is a frontal view of the left and right postcentral gyri (red) together with the WM connections innervating S1. WM tracts connecting the postcentral gyri to the corticospinal tracts are drawn in bright green and highlighted by green arrows (see *Results* section for details).

Table

Demographic information for the IBS and HC samples (see Methods section for the key to the abbreviations). Listed are the mean μ and standard deviation σ of the HAD (Hospital Anxiety and Depression) and STAI (State Trait Anxiety Inventory) tests, Bowel Symptom Questionnaire (BSQ), and PHQ (Patient Health Questionnaire), including the PHQ-15, PHQ-13 (no IBS-related questions), and the PHQ-12 (no GI-related questions). Additionally reported in the rightmost three columns are Welch's *t* statistic (for comparing two samples of unequal sizes and variances), the degrees of freedom (d.f., approximated from the Welch-Satterthwaite equation), and the *p* value (uncorrected).

		IBS		HC		statistics		
	measure	**μ**	**σ**	**μ**	***σ***	***t***	**d.f.**	***p***
test	BH	3.18	2.11	4.00	0.00	−2.22	32.00	0.027*
	BMI	26.74	4.63	24.09	3.29	2.88	51.22	0.002
HAD	anxiety	4.88	4.51	3.14	2.48	2.03	43.65	0.024
	depression	2.64	2.80	1.13	1.31	2.91	40.35	0.003
STAI	anxiety	37.16	12.53	27.57	7.65	3.98	46.30	<0.001
PHQ	15	8.21	3.21	1.41	1.34	11.41	40.93	<0.001
	13	5.12	2.93	1.34	1.42	6.94	40.98	<0.001
	12	3.88	2.84	1.27	1.38	4.95	41.13	<0.001
BSQ	overall score	10.63	4.76	—	—	—	—	—
	abdominal pain	9.94	4.52	—	—	—	—	—
	bloating	11.18	5.20	—	—	—	—	—
	usual IBS severity	3.21	0.60	—	—	—	—	—
	IBS onset age (yrs)	23.94	9.70	—	—	—	—	—
	IBS duration (yrs)	13.68	10.66	—	—	—	—	—

* Student's *t* test was applied in this case to determine whether the BH score of the IBS patients differed significantly from that of HCs, which was equal to 4 (normal). Because the standard deviation of the BH score for HCs is zero, Student's *t* test is more appropriate in this case because it allows one to investigate whether the mean BH of the IBS patients is significantly different from a reference value of 4, as in one-sample *t* test.