Understanding Neurogastroenterology From Neuroimaging Perspective: A Comprehensive Review of Functional and Structural Brain Imaging in Functional Gastrointestinal Disorders

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This review provides a comprehensive overview of brain imaging studies of the brain-gut interaction in functional gastrointestinal disorders (FGIDs). Functional neuroimaging studies during gut stimulation have shown enhanced brain responses in regions related to sensory processing of the homeostatic condition of the gut (homeostatic afferent) and responses to salience stimuli (salience network), as well as increased and decreased brain activity in the emotional response areas and reduced activation in areas associated with the top-down modulation of visceral afferent signals. Altered central regulation of the endocrine and autonomic nervous responses, the key mediators of the brain-gut axis, has been demonstrated. Studies using resting-state functional magnetic resonance imaging reported abnormal local and global connectivity in the areas related to pain processing and the default mode network (a physiological baseline of brain activity at rest associated with self-awareness and memory) in FGIDs. Structural imaging with brain morphometry and diffusion imaging demonstrated altered gray- and white-matter structures in areas that also showed changes in functional imaging studies, although this requires replication. Molecular imaging by magnetic resonance spectroscopy and positron emission tomography in FGIDs remains relatively sparse. Progress using analytical methods such as machine learning algorithms may shift neuroimaging studies from brain mapping to predicting clinical outcomes. Because several factors contribute to the pathophysiology of FGIDs and because its population is quite heterogeneous, a new model is needed in future studies to assess the importance of the factors and brain functions that are responsible for an optimal homeostatic state.
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Key Words
Dyspepsia; Homeostasis; Irritable bowel syndrome; Neuroimaging; Visceral pain

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Introduction

Under stress conditions, gastrointestinal (GI) symptoms such as nausea, diarrhea, and abdominal pain are common, so a close interaction between the brain and the gut has long been assumed.[1-4] The availability of brain imaging technology from the 1990s has allowed exploration of the central control of human gut function in health and disease.[2] Initial neuroimaging studies using positron emission tomography (PET) reported rectal distention[5] and esophageal distention,[6] followed by gastric distention.[7] Brain imaging research has grown rapidly over the past 2 decades; in particular, various neuroimaging modalities have contributed to our increased understanding of the complex bidirectional interaction between the brain and gut (gut and brain) in functional gastrointestinal disorders (FGIDs). The variety of neuroimaging techniques are summarized in Table. This review provides a comprehensive overview of the brain imaging studies in FGIDs to encourage the understanding of researchers other than brain image experts.

Functional Gastrointestinal Disorders and Brain Imaging (What Are Functional Gastrointestinal Disorders? Why Is Brain Imaging Useful?)

FGIDs are characterized by chronic pain, discomfort, and other general symptoms in various locations within the GI and biliary tracts that occur without an apparent physical, biological, or anatomical etiology.[1] They encompass more than 40 different heterogeneous disorders, including functional dyspepsia (FD), functional chest pain, and irritable bowel syndrome (IBS). They are remarkably common diseases, eg, the prevalence of FD and IBS are 10.0-30.0%[8] and 11.2%[9] worldwide, respectively. It is well known that FGIDs tend to feature comorbid affective disorders such as anxiety, depression, and somatization,[10] and their start and development are highly influenced by stress.[11] One difficulty in managing symptoms in FGIDs is that there is no identifiable cause or biomarker for diagnosis. To better understand the pathophysiology of FGIDs, authorities such as the Rome committee have proposed a biopsychosocial model with integrated biological, psychological, and social subsystems interacting at multiple levels.[1] FGIDs are now defined as gut–brain interaction disorders classified by GI symptoms related to any combination of the following: motility disturbance, visceral hypersensitivity, altered mucosal and immune function, altered gut microbiota, or altered central nervous system processing.[11-13] Imaging the brain substantiates the patient's subjective reports of their symptoms, eg, visceral pain (the key feature of FGIDs), and the

initial studies with PET visualized visceral pain associated activated patterns in the brain via esophageal, gastric, and rectal stimulation. These studies evaluated the influence of emotional and cognitive factors on the perception of afferent gut signaling. Conversely, information about the gut environment or chronically enhanced gut signaling may induce transient or long-term neuroplasticity changes in the brain that cause central sensitization as well as affective changes such as anxiety and depression. The most unique aspect of brain imaging studies in neurogastroenterology is the assessment of the correlation between the brain and peripheral functions, such as GI sensation, motility, and immune function, and gut microbiota compositions within the framework of the biopsychosocial model, which takes into account the influences of environmental, psychological, and biological factors on the interaction.

Brain-Gut Interaction: Functional Neuroanatomy of Key Concept of Functional Gastrointestinal Disorders

The brain (central nervous system) and gut (enteric nervous system) have bidirectional interactions that regulate physiological functions and maintain an organism's overall homeostatic state.[3] Afferent visceral signals are conveyed from the gut to the brain via the vagal and spinal pathways.[14] The vagal and spinal splanchnic and pelvic afferent terminals convey the physiological condition including not only mechanical and chemical signals but also microbiota, immune, or endocrine signals originating from the gastroenterological tract.[15] Many afferent signals go unperceived and are used in reflexes that control motility, secretion, blood flow, and other aspects of GI function. The primary vagal and spinal afferents project to the nucleus of the solitary tract in the medulla and lamina I of the dorsal horn, respectively. Signals from both afferent types are integrated in the parabrachial nucleus (PB) within the brainstem, which provides an integrated signal to regulate regions including the forebrain as well as the hypothalamus and amygdala.[3] The visceral afferent information is then routed to the thalamus, where 2 parallel streams of information reach the insula and anterior cingulate cortices (ACC). In the brain, visceral afferent signals are processed in regions related to: (1) the perception of the homeostatic condition of the gut (eg, brainstem sensory nuclei, thalamus, and posterior insula); (2) accompanying emotional arousal reactions (eg, locus coeruleus [LC], amygdala, subgenual and pregenual ACC, and hippocampus); (3) the salience network (eg, anterior insula, anterior midcingulate cortex, and amygdala) that is engaged in response to salient stimuli not limited to pain; and (4) the descending pain modulation system (eg, periaqueductal gray [PAG] and rostroventral medulla), which receives direct nociceptive information from the spinoreticular path-

Table. Brain Imaging Techniques

Implement	What it measures	Utilities in gastroenterology
PET	Measures the annihilation photons from positron-electron annihilation. The positron is emitted from the radioactive tracer that is injected intravenously.	
$[^{15}O]H_2O$ PET	Measures regional cerebral blood flow	Initial studies during gut stimulations.
$[^{18}F]FDG$ PET	Measures the regional metabolic rate of glucose	Used mainly for diagnosis and staging of cancers but also for neurological studies
Relevant radioligands	Neurotransmitter system (dopamine, serotonin, opioids, cannabinoid etc)	Studies are limited due to the high cost, the limited availability of relevant ligands, and the complexity of the studies.
MRI	A non-invasive technique to assess brain function based on endogenous magnetic properties,	
fMRI (BOLD signal)	Measures changes in the proportion of oxygenated vs deoxygenated haemoglobin, which is seen in areas of greater neural activity	Most frequently used in particular task based (eg, gut stimulation) studies. More available and better temporal resolution than PET
ASL	Measures cerebral blood flow directly, by using arterial blood as an endogenous tracer.	Suitable to measure baseline cerebral blood flow.
rsfMRI	Measures spontaneous, low frequency (< 0.1 Hz) fluctuations in the BOLD signal that occur when a subject is not performing an explicit task. rsfMRI investigates synchronous activations between regions that are spatially distinct, occurring in the absence of a task or stimulus, to identify resting state networks.	Several studies used rsfMRI to investigate the resting state networks between healthy controls and patients in a certain disease condition.
MR spectroscopy	Quantitative measure of biochemical concentration in the living brain based on the unique MR spectra of different molecules.	Proton MR spectroscopy can reliably detect metabolites such as Glx (Glutamate and Glutamine), γ-aminobutyric acid (GABA), and N-acetylaspartate (NAA).
Structural MRI	T1 weighted high-resolution structural MRI used to produce structural imaging. Dynamic alterations in brain structure have been observed even within 5 days.	Assess baseline differences between groups and the central nervous system effects of treatments, aging, and disease.
VBM (gray matter)	Whole brain analysis of the density and volume of gray matter in each voxel, which may involve changes in glial number, dendritic spines.	Influence of environmental factors such as early life event on brain structures.
Diffusion imaging	Evaluates white matter integrity and anatomy. The tract integrity is expressed commonly as fractional anisotropy and specific fiber tracts between brain regions are identified by tractography.	Diffusion imaging studies in stroke patients are useful as it shows dynamic remodelling of white matter tracts. Longitudinal studies in therapeutic intervention.
MEG	Measures the magnetic field generated by the electrical activity of neurons with millisecond temporal resolution.	MEG is used to measure the time courses of brain activity (eg, due to gut stimulation). Not widely available
EEG	Measures direct electrical activity of the brain by surface scalp electrodes with millisecond temporal resolution.	EEG is used for evoked potentials to external stimuli in real time or to measure the time courses of brain activity.

PET, positron emission tomography; fMRI, functional magnetic resonance imaging; BOLD, blood oxygen level-dependent; ASL, arterial spin labelling; rsfMRI, resting-state functional magnetic resonance imaging; MR, magnetic resonance; VBM, voxel-based morphometry; MEG, Magneto-encephalography; EEG, electro-encephalography.

way and top-down modulation from the prefrontal executive control and emotional arousal areas of the limbic system and modulates spinal dorsal horn neuron sensitivity.[15] Of note, central structures that process visceral sensory signals not only share the structure of the descending modulatory pathway but also have strong connections to the emotional arousal areas and autonomic and endocrine stress

response structures.[15] Figure 1 shows the brain areas that process visceral afferent signals.

Important mediators of output signals from the brain to the gut are the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis, which are also the main regulators of the stress response.[16,17] Physiological stress such as pain and psychological stress may increase the sympathetic and decrease the parasympathetic tone in the ANS.[18,19] It also upregulates the HPA axis and corticotrophin-releasing hormone (CRH) levels,[18,20] which activates local inflammatory processes and stimulates circulating cytokines that affect gut function, including motility, secretion, epithe-

lial permeability, immune function, and microbiota composition.[15] These changes in peripheral function, in turn, increase peripheral sensitivity to pain[15] and alter and amplify visceral afferent signaling to the brain, which may change visceral pain processing, stress regulatory responses, and/or long-term neuroplasticity changes in the brain.[3,21]
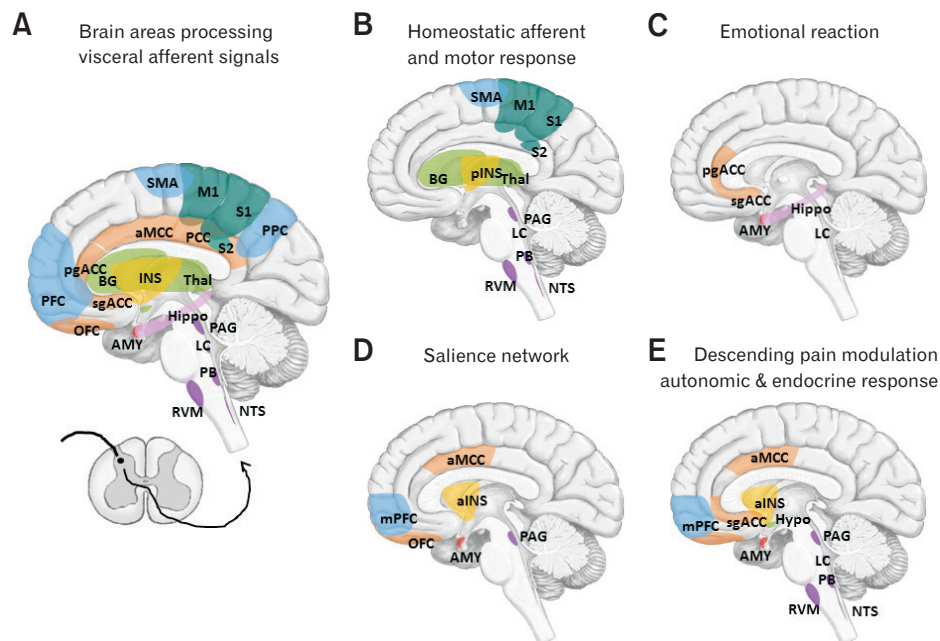

Figure 1. Brain areas processing visceral afferent signals (A) and the areas in which altered brain activity is reported in FGIDs (B-E). (A) Afferent visceral signals conveyed from the gut project to the nucleus of the solitary tract (NTS) and the lamina I of the dorsal horn and are integrated in the parabrachial nucleus (PB) in the brainstem and routed to the thalamus (Thal), where 2 parallel streams of information reach the insula (INS) and anterior cingulate cortices (ACC). (B) Homeostatic afferent: the brain areas related to the sensory processing of the homeostatic condition of the gut are brainstem sensory nuclei (NST and PB), Thal, posterior insula (pINS), and somatosensory cortex (S1 and S2). The basal ganglia (BG), supplementary motor cortex (SMA), primary motor cortex (M1), brainstem nucleus (periaqueductal gray [PAG], locus coeruleus [LC], and rostroventral medulla [RVM]) are associated with preparation of the reaction and motor response to the afferent signals. (C) Emotional reaction: The areas associated with accompanying emotional arousal reactions are the LC, amygdala (AMY), subgenual and pregenual ACC (sgACC and pgACC), medial prefrontal cortex (mPFC), and hippocampus (Hippo). The AMY is a prototypical emotion-related structure with the hippocampal network. The ACC is a multifunctional structure, the sgACC is the principal site of autonomic regulation, and the pgACC is activated in a variety of emotional states. (D) Salience network[16,24]: this network is engaged in response to salient stimuli but not limited to pain. Core region of salience network is the anterior insula (aINS) and anterior midcingulate cortex (aMCC). The midcingulate cortex, part of the dorsal ACC, is a multifunctional region involved in the executive control of attention. The aINS is essential for the conscious experience (bodily) feelings. (E) Descending pain modulation system: endogenous descending pain modulation structures include the PAG and the RVM, which receives direct nociceptive information from the PB and the spinoreticular pathway and top-down modulation from the prefrontal executive control areas (mPFC and dorsolateral PFC [DLPFC]), aMCC and emotional arousal areas (aINS, sgACC, and AMY) and modulates the sensitivity of spinal dorsal horn neurons. These areas are highly connected with autonomic and endocrine response structures (hypothalamus [Hypo] and brainstem nucleus including NTS).

Functional Neuroimaging: Task Based Studies (During Gut Stimulation and/or Cognitive/Emotional Task)

Visceral Afferent Signal Processing

The brain regions consistently activated during rectal, colonic, and gastric distention include the anterior and posterior insula, ACC, primary sensory cortex, prefrontal regions, posterior parietal cortex, and thalamus.[21-23] Whereas the insula and ACC were the most commonly reported regions, primary and secondary sensory cortices and the primary motor cortex were more often reported by studies of the upper GI tract.[22] These brain areas are similar to the regions activated by somatic pain and the so-called "pain matrix" commonly activated by nociceptive stimuli.[24] However, the pain matrix was recently reconceptualized as part of the salient network that is involved in detecting, orienting attention toward, and reacting to salient sensory stimuli, because the majority of brain imaging studies showed activation of the same regions regardless of their stimulus modality.[21,24] In health, it may work as alarm signals on physiological conditions of the viscera; however, enhanced (or modified) activation of the salience network may underlie the symptoms of (functional) GI diseases. Meta-analyses of rectal distention confirmed the above results in both healthy controls (HC) and IBS patients.[25,26] However, these patients showed stronger brain activation than did HC in regions associated with emotional arousal, including the pregenual ACC and amygdala, as well as in regions involved in endogenous pain inhibition and lower activation in the medial and lateral prefrontal cortex, possibly related to weaker engagement of the cortico-pontine-spinal pain inhibition system (Fig. 2).[25] Similarly, the visceral-afferent areas in patients with FD became activated at significantly lower intra-gastric pressures than those in HC.[27] These differential brain activities during visceral afferent signaling in FGIDs indicate that patients may have enhanced visceral afferent signal perception, unappropriated accompanying emotional arousal reactions, and weaker top-down modification of visceral signals. Of note, most studies of rectal distention were performed at the same degree of rectal distention in HC and patients. This produced stronger pain sensations in IBS patients. One paper reported that brain responses to distension were similar between normosensitive IBS patients and HC and that hypersensitive IBS
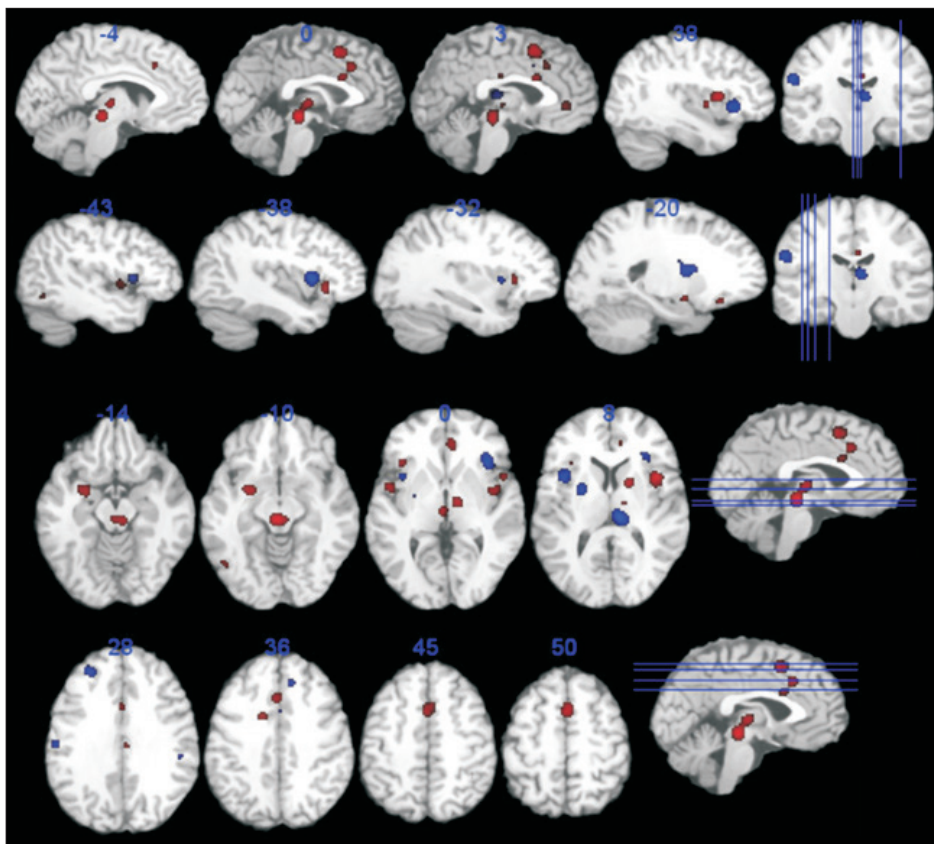

Figure 2. Brain areas demonstrating more (red) or less (blue) activation in irritable bowel syndrome compared to healthy control in a meta-analysis of rectal distention. Adapted from Tillisch et al[25] with permission.

patients had greater activation of the insula and smaller deactivation in the pregenual ACC during noxious rectal distension.[28] This may mean that the difference in brain responses between HC and IBS with visceral hypersensitivity may not be the specific feature of the intracerebral processing of IBS itself but rather the reflection of differences in the thresholds of the sensory, ie, primary afferent or dorsal horn, neurons.

Affective Factors on the Processing Visceral Afferent Signals

In HC, negative emotional induction by facial or music increased activation of the brain regions associated with the processing of visceral afferent signals including the ACC and insula, as well as increased ratings of anxiety and discomfort during esophageal sensation/pain.[29,30] Public speaking stress enhanced activation in the posterior cingulate cortex (PCC) and somatosensory cortex during a rectal distention paradigm.[31] On the other hand, fatty acid infusion into the stomach attenuated activation of the pons, hypothalamus, and hippocampus during sad emotional induction.[32] HC with a higher neuroticism score showed higher activity in the salience network and the parahippocampal gyrus during pain expectation and lower activity in the same areas during actual pain as a result of esophageal stimulation.[33] In IBS patients compared with HC, anxiety-induced modulation of the neural response to rectal distention was stronger in the insula, ventrolateral prefrontal cortex (VLPFC), and anterior midcingulate cortex (aMCC). In contrast, the modulation of the dorsolateral prefrontal cortex (DLPFC) activation in an anxiety context was reduced, which indicates impaired recruitment of the endogenous mechanism of pain control.[31] In association with anxiety, FD patients showed no activation in the pregenual ACC (pgACC), no deactivation in the dorsal pons during distension, and no deactivation in the amygdala during sham treatment.[34] This may represent arousal—anxiety-driven failure of pain modulation in FD.[34] A history of early adverse life events (EALs) enhances MCC and PCC activation, decreases subgenual ACC (sgACC) activation, and leads to stronger rectal distention-induced pain reporting in IBS patients.[35] Activation of the pain modulatory regions (dorsolateral and dorsomedial prefrontal cortices [PFC]) and amygdala is diminished during gastric distention.[27] It is well known that EALs are more prevalent in IBS and associated with an increased HPA axis response[36] and epigenetic changes.[37,38] EALs may influence direct or indirect brain processing of visceral afferent signals. Emotion, personality, and EALs influence the brain's processing of visceral signals from the gut and vice versa through direct or indirect mechanisms including expectation and genetic or neurohormonal modulation.

Cognitive Effect on the Processing of Visceral Afferent Signals

Cognition *per* se is a function of brain activity. Several trends of the psychological states are known to affect the processing of the visceral afferent signals.

Anticipation of visceral pain

Patients with IBS, compared with HC, demonstrated less anticipatory inhibition in the insula, sgACC, amygdala, and dorsal brainstem and showed increases in brain activity in the dorsal ACC and dorsal pons during rectal distention.[39] Uncertain anticipation induced stronger MCC activation during the anticipation phase and in the MCC, PCC, and precuneus during subsequent rectal stimulation.[40] The anticipation of visceral pain is considered preparatory for cognitive coping strategies that affect the neural processing of the visceral sensation itself. FGID patients may show failure of this preparatory modulation.

Conditioning with visceral stimulation

A Pavlovian conditioning paradigm with visceral stimulation can be used to study the influence of anticipation on visceral pain experience. Brain regions involved in the perception of visceral pain (esophageal and rectal) were activated when pain was conditioned by a cue (visual stimulus) but not actually delivered. This shows that a normal sensory experience may be exaggerated by the conditioning mechanism.[41] IBS patients compared to HC showed stronger activation in the PCC and ventrolateral PFC (hypervigilance) and amygdala (pain-related fear) as well as enhanced activation in the ventromedial PFC and lateral orbitofrontal cortex (OFC) to the safety cue during the acquisition phase.[42] During reinstatement, they showed enhanced PCC activation (greater attention) during extinction and stronger hippocampal activation (memory reactivation).[42] Patients with IBS may be more hypervigilant and apt to engage attention in the associative learning and memory of abdominal pain.

Placebo: expectation of treatment

Placebo designs have demonstrated the effect of negative and positive expectation of treatment on the brain processing of visceral afferent signals. A positive expectation demonstrated greater decreases in activation of the pain modulation areas (DLPFC), pain sensory areas (insula, ACC, sensory cortex, and thalamus), and amygdala.[43,44] Negative expectation by nocebo instruction increased activation in sensory regions during anticipation (eg, somatosensory

cortex and amygdala) and during painful rectal stimulation (thalamus, insula, and amygdala). IBS patients demonstrated reductions in the placebo effect in pain-processing areas such as the thalamus, somatosensory cortices, insula, and ACC.[45-47] IBS might be characterized by impaired cognitive pain modulation. Of note, patients with inflammatory bowel disorder did not show impaired placebo neural response like those with IBS, which suggests the specificity of this finding for IBS.[47]

Furthermore, a reduction in the effective connectivity of the central executive network circuitry (including the parietal and dorsal lateral PFC) during repeated exposure to a threatening GI stimulus[48] and decreased DLPFC in relation to weaker error feedback function during the Wisconsin Card Sorting Test (WCST) in IBS[49] support reduced cognitive modulation in patients with FGIDs.

Descending Modulation of Visceral Afferent Signals

Two studies demonstrated that diffuse noxious inhibitory control is dysfunctional in IBS patients compared with HC suggesting dysfunction of spinal and/or supraspinal endogenous pain modulation mechanisms in IBS patients.[3,50]

Neuroimaging Studies of Mediators of Brain-Gut Interaction

Stress mediators of the brain-gut interaction such as ANS and HPA axis function have been studied repeatedly in FGIDs.[19,51] However, studies of the dynamic interaction between the brain and the gut and its circular loop as well as its interaction with other factors as a whole system remain sparse. Farmer et al[52] examined the existence of distinct human pain clusters composed of discrete psychobiological and genetic profiles linked with variations in pain perception and processing. Patients in cluster 1 had higher neuroticism, greater baseline sympathetic tone, and higher cortisol release, an over-representation of the short allele of the serotonin transporter-linked polymorphic region (5-HTTLPR); an increased parasympathetic response to pain; and lower pain thresholds compared with patients in cluster 2, who had the opposite profile. Patients in cluster 1 demonstrated greater activity in the left frontal cortex, whereas patients in cluster 2 showed greater activity in the right medial/frontal cortex and anterior insula during esophageal distention.[52] The genetic effect of 5-HTTLPR on brain-gut interactions has also been reported. Colorectal distention in individuals with the s/s genotype activates the ACC, hippocampus, and OFC more than that in individuals with the l allele.[53] Another study examined the effects of CRH injection on colonic motility, ANS activity, and endocrine responses as well as brain activity alterations between IBS patients and HC. In brain responses to rectal distention, a negative association between the ACTH response to CRH and activity in the pgACC was identified in HC but not in IBS patients. This study indicates impaired top-down inhibitory input to the HPA axis from the pgACC in IBS.[54]

Sex-based Differences

In HC and patients with FGIDs, greater engagement of the emotional brain circuits was found in females versus greater engagement of the prefrontal circuits in males. During esophageal stimulation, women showed stronger activation in the midcingulate cortex, the affective component of pain.[55] During the expectation of rectal pain, stronger coupling was observed among emotional arousal regions in female than in male patients with IBS.[56] However, it must be emphasized that the published literature on sex differences in brain activation by visceral stimuli is still sparse and somewhat contradictory. Although many reviews emphasized the importance of considering sex-related differences, no conclusive studies have been performed.

Resting-state Functional Magnetic Resonance Imaging

Brain activity is present even in the absence of an externally activated task and causes fluctuations in blood oxygen level-dependent (BOLD) signals. Resting-state functional magnetic resonance imaging (rsfMRI) measures the spontaneous fluctuations during rest and uncovers the intrinsic brain functional architecture, ie, functional connections of specific brain regions and local networks as well as overall organization of functional communication in the brain network (Fig. 3A).[57,58] Even at rest, the brain's functional networks that work together during tasks continue to harmonize along with their own distinguishable frequencies and phases and provide a signature of the functional organization. Alterations of the intrinsic networks may represent endophenotypes of disease vulnerability.

Several methods have been proposed to study functional connectivity using rsfMRI. Seed-based analysis correlates the resting-state time series between a seed region of interest and all other voxels in the brain. Independent component analysis (ICA) are model-free data-driven methods that determine spatial component maps of resting-state signals that are maximally independent of each other. In contrast, to detect correlations in fluctuations of the BOLD signals between regions or the components by ICA, the amplitude of low-frequency fluctuations (ALFF) estimates the amount of low-
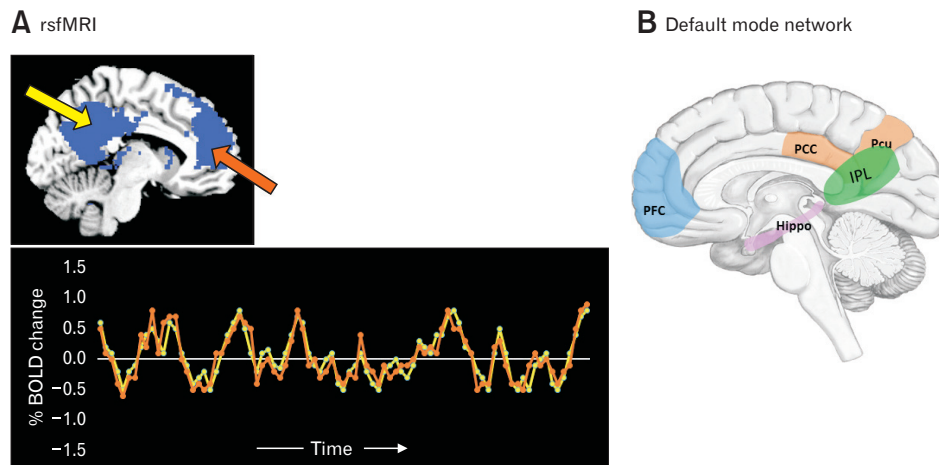
A rsfMRI

B Default mode network

Figure 3. Resting-state functional magnetic resonance imaging (rsfMRI) and default mode network. (A) rsfMRI is used to investigate synchronous neural activity (as measured with the blood oxygen level-dependent [BOLD] signal) between spatially distinct brain regions and provides the functional architecture of the brain. The lower panel represents the synchronous fMRI BOLD signal activity from the posterior cingulate cortex (yellow arrow in the upper panel) and in the medial prefrontal cortex (orange arrow in the upper panel). Adapted from Raichle.[58] (B) Default mode network (DMN): the set of areas that work together at rest and are involved in high-level cognitive processes such as self-awareness and memory. DMN is thought to consist of the medial prefrontal cortex (mPFC), posterior cingulate cortex (PCC), hippocampus (Hippo), superior temporal gyrus, inferior parietal lobule (IPL), and precuneus (Pcu).

frequency power in each voxel in the brain.[59] Regional homogeneity (ReHo) calculates the correlation of a voxel's time series with that of its local neighboring voxels and focuses on localized short-distance connectivity.[59] A graph theoretical analysis was recently proposed to study the topological characteristics of complex networks that can quantify the global organization of the network as well as the local functional characteristics of this brain network.[57,60]

Use of Resting-state Functional Magnetic Resonance Imaging in Irritable Bowel Syndrome

Studies using rsfMRI in IBS/FD began in the early 2010s; since then, several studies have investigated group differences of resting-state functional connectivity or correlation between parameters of rsfMRI and clinical indices. However, analysis methods of rsfMRI varied across studies and their results demonstrated considerable diversity.

Seed-based analysis

A bilateral dorsal anterior insula seed-based analysis reported a negative functional connectivity of the dorsal anterior insula with the medial PFC (mPFC) and precuneus in female IBS patients compared with female controls. Furthermore, a GI-specific anxiety score measured by the visceral sensitivity index was significantly correlated with functional connectivity between the dorsal anterior insula and the dorsal mPFC in male IBS patients and the precu-

neus in female IBS patients.[61] An amygdala seed-based analysis showed a higher functional connectivity between the amygdala and insula, midbrain, and sensorimotor regions in IBS versus HC.[62]

Independent component analysis

Icenhour et al[63] reported that resting-state functional connectivity value in the default mode network (DMN) (Fig. 3B) and the sensorimotor network was associated with rectal perception thresholds, and that the resting-state functional connectivity value in the posterior insula was correlated with the reported symptom severity in IBS.[63]

Amplitude of low-frequency fluctuations

Hong et al[61] reported that female IBS subjects had a frequency power distribution skewed toward higher frequencies in the insula and toward lower frequencies in the sensorimotor cortex to a greater extent than in male IBS subjects. The skew toward higher frequencies in the anterior insula was positively correlated with symptom-related discomfort.[61] Another study showed decreased ALFF values in the left superior frontal gyrus, right hippocampus, right middle frontal gyrus, bilateral postcentral, and right superior temporal pole, as well as increased values in the left middle cingulate and left calcarine gyrus in IBS patients.[64] Qi et al[65] reported that IBS patients showed decreased ALFF in several core DMN regions (mPFC, PCC, and bilateral inferior parietal lobule [IPL]), but increased

ALFF in the bilateral posterior insula and cuneus. Additional seed-based analyses demonstrated differences in some inter-regional functional connectivity between IBS and HC.[65]

Regional homogeneity

IBS patients showed increased ReHo in the postcentral gyrus and thalamus and decreased ReHo in the ACC and PFC.[66]

Global organizations

Weng et al[67] calculated functional connectivity density (FCD) for long- and short-range FCD values. They found that, compared with HC, IBS patients showed decreased long and short FCD in bilateral aMCC and IPL, decreased long FCD in the right anterior insula, and decreased short FCD in the bilateral PFC, sACC, and caudate. The abnormal FCD values in the right anterior insula and left caudate were correlated with IBS severity and disease duration, respectively.[67] A graph analysis of the DMN showed decreased global efficiency in IBS patients.[68]

Use of Resting-state Functional Magnetic Resonance Imaging in Functional Dyspepsia

Seed-based analysis

Compared with HC, FD patients had increased connectivity between PAG and the insula and decreased connectivity between the PAG and the OFC, DLPFC, and hippocampus/parahippocampus. Highly anxious and depressed FD patients had altered PAG connectivity with the ACC, precuneus, DLPFC, and caudate.[69] FD patients had higher global interhemispheric functional connectivity than HC, and voxel-wise analysis showed increased interhemispheric functional connectivity in the ACC insula and thalamus.[70]

Independent component analysis

Liu et al[71] used ICA and reported an alteration in the DMN in FD, and dyspepsia symptom severity was positively correlated with the functional connectivity value in pgACC and negatively correlated with the functional connectivity value in OFC.[71]

A multivariate analysis was used to classify FD patients from controls based on functional connectivity and showed that abnormal functional connections were mainly within or across the limbic/paralimbic system, PFC, temporoparietal areas, and visual cortex. Furthermore, the classification features were significantly associated with the patients' dyspepsia symptoms and depression and anxiety scale scores.[72]

Amplitude of low-frequency fluctuations

Zhou et al[73] estimated fractional ALFF (fALFF), the ratio of the power spectrum of low frequency to that of the entire frequency range, and reported group differences in patients with FD who had increased fALFF in multiple regions, including the insula, brainstem, and bilateral cerebellum. The fALFF in the insula was positively correlated with disease severity. Additional seed-based analyses revealed increased functional connectivity between the right cerebellum and multiple brain regions.[73]

Regional homogeneity

Liu et al[74] applied a multivariate analysis and showed highly discriminative brain regions between FD and HC based on ReHo, mainly in the PFC, OFC, supplementary motor cortex, temporal pole, insula, ACC/MCC, thalamus, hippocampus, parahippocampus, and cerebellum. ReHo values were positively correlated with symptom severity in the dorsal mPFC and pgACC, whereas they were correlated with FD disease duration in the MCC, OFC, insula, and temporal pole.[74] Nan et al[75] found altered ReHo values in multiple brain areas in FD patients. ReHo values in the ACC and thalamus were correlated with symptom severity of dyspepsia.[75]

Although there is considerable variety in methodology, these results may indicate abnormal functional connectivity in IBS or FD. At the local level, abnormal resting-state functional connectivity was reported, mainly in pain-related areas (anterior insula, ACC, amygdala, and sensory motor cortex) and the DMN (PCC, precuneus, mPFC, and hippocampal complex).

Structural Magnetic Resonance Imaging ——

Brain Morphometry

Cortical thickness and gray matter density

High-resolution structural MRI measures the brain anatomy and can be used to quantify and systematically compare morphological differences in brain structures.[76] The most commonly used method is voxel-based morphometry, images of which provide a measure of gray-matter density or volume. Variations of these measurements may reflect differences in cell types, neuron densities, dendritic harboring, and other causes.

Irritable Bowel Syndrome

IBS patients have increased gray matter density (GMD) in the hypothalamus and increased cortical thickness in the aMCC.

A negative correlation between the pain catastrophizing scale and DLPFC thickness and a positive correlation between pain duration and anterior insula thickness is seen in these patients.[77] Seminowicz et al[78] reported that IBS patients showed increased GMD in the pgACC and OFC and a decreased GMD in the mPFC, DLPFC, PCC, ventral striatum, and thalamus. However, the group differences remained only in the PFC and PCC when anxiety and depression levels were controlled.[78] Another study showed that female IBS patients compared with female controls showed increased cortical thickness in the pre- and post-central gyrus and decreased thickness in the bilateral insula and the left subgenual ACC (sgACC).[79] Lower gray matter volumes (GMV) in the insula, cingulate, amygdala, hippocampus, putamen, and frontal regions and higher GMV in the left postcentral gyrus were observed in female IBS patients compared with HC. However, many of the differences were accounted for by histories of early trauma.[80] Further, they applied the graph theory and found no group differences in global and local network organization.[80] In an experiment of the combination of a pain modulation examination using electrical stimulation and heterotopic noxious counter-stimulation, a greater thickness in the right posterior insula was associated with a longer disease duration in IBS, while a thicker right lateral OFC was associated with less pain inhibition in both IBS patients and HC.[81] Rectal sensitivity was associated with GMV in the thalamus, insula, PCC, VLPFC, OFC, amygdala, and basal ganglia and negatively associated with GMV in the right thalamus.[82] Catecholaminergic gene polymorphisms are associated with morphological alterations in IBS patients.[83] Labus et al[84] performed a multivariate analysis of morphometric data (volume, mean curvature, surface area, and cortical thickness) to identify potential brain endophenotypes that discriminate IBS patients from HC.[84] Although associations between the brain signatures and clinical measures were weak, alterations in sensorimotor regions dominated the morphometry based classifier, which share similar results in other chronic functional pain disorders.[84]

Functional Dyspepsia

Patients with FD with postprandial pain compared to HC showed a decreased GMD in the bilateral precentral gyrus, mPFC, ACC, MCC, left OFC, and right insula. When considering anxiety and depression, the reduced GM density in the bilateral middle frontal gyrus, left MCC, right precentral gyrus, and insula remained. The GMD decreases in the ACC were significantly associated with symptom scores.[85] On the other hand, Liu et al[86] found that FD patients had a higher GMV in the bilateral putamen and right caudate, and additional seed-based structural covariance patterns demonstrated that FD-related differences were mainly located in the amygdala, hippocampus/parahippocampus, thalamus, lingual gyrus, and cerebellum. In these patients, significant positive correlations were found between the volumes in the striatum and the FD disease duration.[86] Another study demonstrated that FD patients exhibited decreased GMD in the right posterior insula, right inferior frontal cortex, and left MCC.[87]

White Matter Microstructure

Diffusion imaging detects white matter (WM) bundles and provides measures of WM integrity. Diffusion MRI measures the displacement of water molecules and is much faster along the WM fibers then perpendicular to them. Diffusion imaging quantifies the diffusion properties of WM.[88] Fractional anisotropy (FA), a frequently used measure, is related to regional WM features such as axon caliber, fiber density, and myelination. Higher FA values indicate more efficient neuronal conduction through the WM. Probabilistic tractography is used to assess the structural connection between two regions of interest.[89]

Irritable Bowel Syndrome

Chen et al[90] extracted mean FA values from WM regions associated with nociception and provided preliminary evidence for fornix and insular WM alterations in IBS patients. Ellingson et al. found that IBS patients had lower FA values in the basal ganglia and sensory/motor association/integration regions as well as higher FA in the frontal lobe regions and corpus callosum.[91] IBS patients had a reduced mean diffusivity within the globus pallidus and a higher mean diffusivity in the thalamus, internal capsule, and coronal radiate projections to the sensorimotor regions. This suggests differential changes in axon/dendritic density in these regions.[91] Using multivariate regression analysis, Irimia et al[92] reported abnormalities in the mean FA of WM connections innervating the viscerotropic portions of the sensory cortex in IBS patients. Another study including a whole-brain voxel-wise analysis demonstrated that IBS patients had reduced FA in the splenium of the corpus callosum, right retrolenticular area of the internal capsule, and right superior corona radiata.[93] Comparing functional constipation, constipation-predominant IBS (IBS-C), and HC, the genu of the corpus callosum showed a negative association with abdominal pain or discomfort intensity in functional connectivity and IBS-C. On the other hand, functional connectivity exhibits more regions of WM changes than IBS-C, such as the corona radiate, which includes projection fibers associated with the corticopontine tract.[94]

Functional Dyspepsia

One paper reported an increased FA along with reduced mean and radial diffusivity in multiple WM tracts in FD with postprandial distress syndrome (FD-PDS) versus HC and the inclusion of anxiety and depression as covariates abolished the group difference except in the corona radiate. The corona radiate contains commissural and cortico-efferent fibers and the results support cortical modulation of the homeostatic reflex at the central nervous system level in FGID patients.[95]

These studies may indicate abnormal WM microstructures in the brain associated with visceral afferent signal processing. However, the results are still inconsistent and further studies are required to reach a final conclusion.

Molecular Imaging

Molecular imaging modalities such as magnetic resonance spectroscopy and PET characterize biological processes at the molecular and cellular levels. Magnetic resonance spectroscopy assesses the levels of biochemical metabolites in the brain such as glutamate/glutamine (Gly) and γ-aminobutyric acid (GABA). PET uses radioligands injected into the bloodstream that have high binding affinity for target molecules. There are few molecular imaging studies in the field of gastroenterology.

Irritable Bowel Syndrome

Niddam et al[96] demonstrated a reduction in hippocampal Gly in IBS patients and that Gly concentrations were inversely related to emotional stress indicators in IBS patients. These findings indicated abnormal functioning of hippocampal glutamatergic neurotransmission and dysfunction of the inhibitory role of the hippocampus in IBS.[96]

A PET study demonstrated that serotonin synthesis was greater in the right medial temporal gyrus in female IBS patients than in female controls.[97] A follow-up study investigated the effect after a 14-day oral treatment with alosetron, a $5\text{-}HT_3$ receptor antagonist, and found that $5\text{-}HT$ synthesis was greater in male IBS patients than in female IBS patients.[98]

Functional Dyspepsia

Mak et al[99] found enhanced glutamate transmission in the somatosensory cortex in FD-PDS linked to post-prandial distress chronicity and severity as well as anxiety. A PET study on FD revealed that the level of the serotonin transporter in the midbrain and thalamus was higher in patients with FD than in HC. This in-

dicates upregulation of the serotonin transporter level in gut afferent signal-related areas in patients with FD.[100] Another PET study of a cannabinoid-1 receptor radioligand demonstrated that FD patients had higher cannabinoid-1 receptor availability in areas related to visceral nociception as well as homeostatic and hedonic regulation of food intake, which may indicate sustained endocannabinoid dysfunction in FD.[101]

Other Studies

Repetitive transcranial magnetic stimulation targeting the right secondary somatosensory cortex in patients with chronic pancreatitis and severe visceral pain revealed a significant analgesic effect that was correlated with an increase in excitatory neurotransmitter levels such as glutamate and N-acetyl aspartate.[102]

Microbiota-Brain Interaction in Functional Gastrointestinal Disorders

Findings in rodents indicate that the gut microbiota may contribute to the pathophysiology of FGIDs[103] as well as brain disorders including autism spectrum disorders,[104] Parkinson's disease,[105] and affective disorders.[106] The burgeoning number of preclinical studies demonstrated that the gut microbiota plays a significant role in brain development,[107] the stress response system,[108,109] and the brain neurotransmitter system.[110,111] However, there is very limited evidence that replicates these animal data in the brains of patients with gut-brain disorders.[110]

Healthy women who consumed dairy products containing probiotic bacteria had a reduced response in a network including the primary interoceptive and somatosensory regions as well as the midbrain regions during an emotional face recognition task.[112] In addition, activity in the interoceptive, affective, and prefrontal regions has changed during resting-state measurements.[112] In healthy women, bacterial genus-based clusters were identified: a cluster with a greater abundance of *Bacteroides* and one with a greater abundance of *Prevotella*. The *Prevotella* group showed less hippocampal activity on viewing negative valence images.[112] Patterns of white and gray matter imaging discriminated between the 2 clusters.[113] In a placebo-controlled trial of IBS patients taking probiotics, *Bifidobacterium longum* reduced responses to negative emotional stimuli in the amygdala and frontolimbic regions and reduced depression scores.[114] Labus et al[115] clustered subgroups of IBS patients based on distinct microbial clusters, but this grouping was rarely correlated with the clinical symptoms of IBS. They also found structural brain alterations, primarily in the sensory integration areas and in

the salience network in correlation with the microbial taxa. This correlation was different between IBS patients and HC.[115] Short-chain fatty acids, which are gut microbiota-driven metabolites produced from fermentable carbohydrates such as acetate, propionate, and butyrate, modulated feeding behavior via central mechanisms in rodents.[116] A diet-associated increase in colonic propionate production and the level of increased propionate were associated with reduced brain activity during high-energy food picture evaluation in the caudate and nucleus accumbens. This finding suggests attenuating reward-based appetite behavior and was independent of changes in plasma peptide tyrosine-tyrosine and glucagon-like peptide 1, glucose, or insulin concentrations.[117] The levels of gut hormones, such as ghrelin, that promote appetite are associated with increased activation in the PFC, amygdala, and insula and decreased activation in the hypothalamus. In contrast, glucose, insulin, leptin, peptide tyrosine-tyrosine, and glucagon-like peptide 1 affect the same brain regions conversely.[118] Although there is some preliminary evidence, neuroimaging studies aiming to prove the preclinical gut microbiota findings have just started and further well-designed translational and clinical studies are required to study the role of microbiota in human health and disease.

Progress of Analysis of Neuroimaging

Shift From Brain Mapping to Predicting Clinical Outcome

Traditional brain analyses focused on brain mapping to identify brain regions responding to a task or showing a different response between HC and patients in a disease condition. In contrast, recent machine learning algorithms or multivariate analyses are used as a data-driven approach to identify brain activity patterns that have specific profiles to predict outcomes. For example, using these techniques, pain levels can be predicted from brain activity patterns or a classifier can be constructed to distinguish IBS from HC based on their brain activity. Using structural MRI data, Labus et al[84] developed a classification model to identify the regions that contribute most to the classification. The model discriminated patients from HC with 70% accuracy and provided a morphological brain signature for IBS patients.[84] Liu and Nan adapted this approach to distinguish patients with FD from HC using rsfMRI.[72,74] Such generalized brain patterns can be used as a brain-based biomarker of the specific disease to assess the effect of pharmacological or therapeutic interventions.

Multicenter Collaborations and Big Data in Neuroimaging

To date, most neuroimaging studies have had a modest sample size (< 50 subjects). However, studies with big neuroimaging data (> 1000 subjects) are ongoing such as the human connectome project, UK Biobank, and ENIGMA consortium.[119] Although the big data approach aims to increase the power of neuroimaging studies, it will also lead to the detection of subtle effects with a small effect size. Machine learning techniques or ICA can be used to determine the most important factors from these data sets. Multiple-center collaborations may contribute to these big data projects; at the same time, they will also increase the variability due to differences in design, scanner, and acquisition settings as well as differences related to the sample population including age, sex, social status, and other population variables.

Connectivity and Modeling of Complex Systems

With regard to brain imaging analyses, initial research methods aimed to estimate regional brain activity that is associated with some contrast, eg, rectal distention versus non-rectal distention. However, brain function changes dynamically in space and time within brain networks and are influenced by visceral information. Some connectivity analyses can assess the causal relationship between regions. Using a dynamic causal model, Aizawa et al[49] revealed that the input from the PFC to the insula was significantly weaker during WCST in IBS patients. Labus et al[120] examined the effect of tryptophan depletion, which decreased brain serotonin levels, on differential connectivity between the ACC, mPFC, amygdala, and pons during rectal distention between HC and IBS patients. Using graph theory, Qi et al[68] demonstrated that IBS patients showed a topological reorganization of the DMN to a non-optimized regularity configuration.

Conclusion and Future Directions

The current paper reviewed neuroimaging studies in the field of FGIDs. Neuroimaging is particularly useful to increase our understanding of the brain-gut/gut-brain interaction dysfunction in FGIDs. Functional neuroimaging studies during GI stimulation have revealed that regional brain activity in regions associated with sensory processing including the thalamus, sensory cortex, and posterior insula and in regions in the salience network such as the anterior insula and dorsal ACC is related to visceral sensations during stimulation in patients with FGIDs at physiologically similar

levels. In addition, altered brain activity is found in areas associated with emotional response including the amygdala and ACC as well as top-down regulation from the PFC. Descending pain inhibitory system failure was also demonstrated. Some studies also showed the dysfunction of brain regulation on the brain-gut interaction by investigating the association between brain activity and HPA and ANS parameters. These studies suggested that in FGIDs, dysfunctional top-down regulation from the PFC and dysfunctional interactions in homeostatic areas like the hypothalamus, amygdala, and nuclei of the brainstem were associated with autonomic responses and pain regulation.

Studies using rsfMR demonstrated that the intrinsic connectivity during rest may differ between HC and patients, whereas gray matter and WM morphometry findings indicated some alterations. However, these results require replication in larger samples.

Recent developments of analytical methods to assess the pathophysiology of FGIDs are promising but the results show complex inter-study variability. Because FGIDs are complex, we must understand their features in several dimensions: within the gut association; within the brain association; brain-gut interactions with its mediators including ANS, HPA, and immune parameters; within a longitudinal time span from genomic predisposition and/or epigenomic change influenced by the early life environment; to alterations accompanying age; and associations among modalities (functional, structural, neurochemical, etc). However, we also need to understand the variability across laboratories, scanners, study designs, methods, and regions and races worldwide. To solve these problems, multicenter studies with multimodal neuroimaging techniques that consider the interaction with peripheral physiological changes using new methods such as deep learning and graph theory may be helpful. However, because so many factors are interacting in FGIDs, the population will be quite heterogeneous and a new model is needed to understand such complex interactions. In future studies, it may be possible to determine whether FGIDs represent a shift from the optimal standard homeostatic state which contributes to the symptoms.

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