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# REVIEWS

## Fundamentals of Neurogastroenterology: Basic Science

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**This review highlights major advances in neurogastroenterology since Rome IV, offering a condensed summary of a comprehensive document to appear in the forthcoming Rome V book. Prepared by an international team of experts, it emphasizes pivotal studies that have deepened understanding of the physiological and pathophysiological mechanisms underlying disorders of gut–brain interaction (DGBI). These disorders are inherently complex and multifactorial, shaped by interactions among neuronal, epithelial, immune, smooth muscle, interstitial, and microbial populations. The review outlines cutting-edge technologies advancing the field and explores key themes, including brain–gut interactions, neurobiology, and neuroplasticity of the enteric nervous system, neuroimmune function, microbiome influences, and abnormalities of the gut–brain axis in DGBI. Risk factors for DGBI are considered, with serotonergic signaling presented as a conceptual framework for linking symptomatology and pathophysiology. Finally, we discuss how these scientific advances can translate into novel therapeutic strategies to improve patient care.**

*Keywords:* Enteric Nervous System; Central Nervous System; Neuroplasticity; Serotonergic Signaling.

Despite the complex mechanisms modulating gastrointestinal (GI) tract function, there has been a rapid expansion in our understanding of the fundamentals of neurogastroenterology since the publication of Rome IV, nearly a decade ago. We are witnessing many major advances in neurogastroenterology, fueled by novel methodologies, including state-of-the-art imaging techniques and cellular and molecular approaches that allow us to manipulate and leverage neuronal and nonneuronal cells to shed light on the basic cellular mechanisms underlying GI function in health and disease, as well as to support new therapeutic advances to treat disorders of gut–brain interaction (DGBI).

The major themes in this review are enteric nervous system (ENS) neurobiology and neuroplasticity, interactions of neurons with immune cells in physiological and pathophysiological states, and the rapidly emerging and critical role of the gut microbiome, with a focus on the bidirectional communication between the brain and the gut microbiota via extrinsic sensory nerves and the immune system. Although the basic underlying mechanisms of sex as a risk factor for DGBI remain incompletely understood, recent advances are discussed. Furthermore, we probe the question of whether fundamental research in neurogastroenterology is getting us any closer to developing new treatment options for patients with DGBI.

## New Technologies Used to Explore the Fundamental Basis of Neurogastroenterology

In humans, an ingested meal traverses many feet, remaining in the GI lumen for days, while successful defecation requires millisecond-level coordination. This remarkable synchronization across broad spatial and temporal scales involves diverse cell populations within the ENS interacting closely with the central and autonomic nervous systems through bidirectional gut–brain communication. To understand how disruptions within this extensive network lead to DGBI, we require integrated approaches from physiology, developmental biology, neuroscience, genetics, and bioengineering applied across time and space.

DGBI are complex, heterogeneous disorders with genetic underpinnings supported by twin studies and familial clustering. Studies have identified disruptive missense

†Deceased.

***Abbreviations used in this paper:*** **5-HT, 5-hydroxytrytamine; ACh, acetylcholine; $CB_1$, cannabinoid-1; CeA, central nucleus of the amygdala; CNS, central nervous system; CRH, corticotrophin-releasing hormone; DGBI, disorders of gut–brain interaction; DRG, dorsal root ganglia; ELS, early-life stress; ENS, enteric nervous system; GABA, γ-aminobutyric acid; GI, gastrointestinal; GPCR, G-protein-coupled receptor; GR, glucocorticoid receptor; HPA, hypothalamic–pituitary–adrenal; IBS, irritable bowel syndrome; ICC, interstitial cell of Cajal; IL, interleukin; ILC, innate lymphoid cell; IPAN, intrinsic primary afferent neuron; $Na_v$, voltage-gated sodium channels; NICU, neuroimmune cell units; NTS, nucleus tractus solitarius; PDGFR$\alpha^+$ cell, Platelet-derived growth factor receptor-α positive fibroblast-like cell; SCFA, short-chain fatty acid; SERT, serotonin reuptake transporter; SSRI, selective serotonin reuptake inhibitor; TPH, tryptophan hydroxylase; TRP, transient receptor potential; VIP, vasoactive intestinal polypeptide.**

**Most current article**



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mutations in subsets of patients, affecting genes critical for epithelial nutrient processing, neuromuscular coordination, and sensory signaling.[1] These discoveries provided crucial insights into biomarkers and therapeutic targets. Advanced sequencing methods, such as single-cell deep sequencing and spatial transcriptomics, combined with large population studies, are uncovering additional genetic contributors and highlighting connections between gastrointestinal and psychiatric disorders.

Although some DGBI subsets may have single-gene mutations, most involve polygenic inheritance and epigenetic regulation. Given significant DGBI patient heterogeneity, further advances in data curation and analysis using artificial intelligence and natural language processing will be essential to effectively link genotypes with clinical phenotypes (endophenotypes) and objective biomarkers such as GI transit and visceral hypersensitivity.

Gut–brain signaling involves a multitude of interactions among diverse cells, including neuronal, epithelial, immune, smooth muscle, and interstitial, as well as microbial populations. Single-cell sequencing has significantly advanced our understanding of these diverse cellular groups.[2] Organoid technologies combined with next-generation sequencing have revealed striking epithelial cell heterogeneity along the crypt–villus axis and throughout the GI tract.[3] Artificial intelligence-driven analyses are increasingly mapping epithelial secretomes and interactomes, whereas microfluidic models recreate native tissue architectures. Similarly, next-generation sequencing technologies have demonstrated that immune cell populations are highly diverse, with distinct lifespans and functions.

Within the ENS, or "second brain," single-cell transcriptomic profiling in rodents and humans has identified substantial neuronal diversity, highlighting both shared subclasses and notable species-specific differences.[4] Approaches including whole-tissue clearing, retrograde tracing, and barcoding have characterized extrinsic gut-innervating sensory neurons. Spatial transcriptomics and integrated imaging methods further elucidate anatomical localization and functional identity of neuronal populations, facilitating detailed cell atlases and circuit maps.[5]

Precise evaluation of gut–brain interactions requires targeted modulation and sensing of specific cells. In addition to knocking in and out genes of interest, genetic engineering methods, such as cre-lox recombination, enable selective expression of genetically encoded sensors, including calcium indicators like GCaMP and emerging sensors for second messengers (cyclic adenosine 5'-monophosphate, voltage). Engineered microbial biosensors now offer diagnostic and therapeutic potential.[6] Optogenetic and chemogenetic approaches, such as DREADDs (Designer Receptors Exclusively Activated by Designer Drugs), have successfully selectively manipulated GI tract cell populations. Further technologies, such as magneto-, sono-, and thermogenetics are promising additional stimulation approaches suitable for the luminal accessibility and constant motility of the GI tract.[7] Nonconventional animal models, such as zebrafish, *Caenorhabditis elegans*, and hydra, have also provided unique insights due to their genetic tractability and comparatively simplified physiologies.[8] Collectively, rapidly evolving technologies continue to revolutionize our understanding of gut–brain physiology and disease, providing powerful tools for dissecting and ultimately treating DGBI (Figure 1).

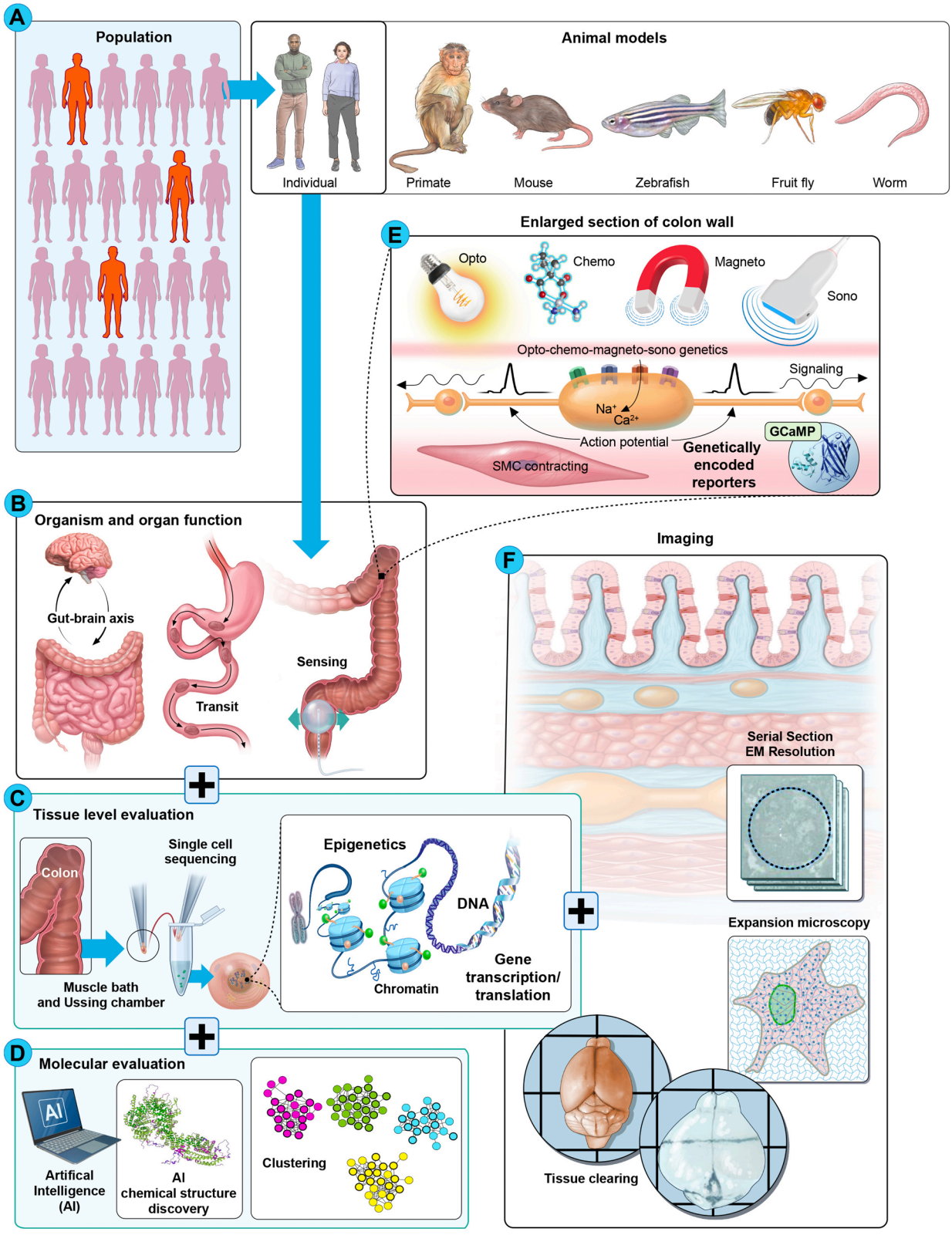
## The Basis of Brain–Gut Interactions

Our ability to detect chemical and mechanical stimuli is essential for responding to environmental challenges. To manage the constantly changing conditions within the GI tract, our body relies on internal surveillance systems. These systems regulate key functions, such as secretion, absorption, and motility, to tightly coordinate and optimize nutrient uptake, fluid balance, and digestion. The GI tract also has crucial barrier and immunologic functions, which include its interface with intestinal microbiota.

To perform these tasks, the GI tract is innervated by diverse populations of neurons. This includes intrinsic neurons (enteric and viscerofugal), whose cell bodies lie within the gut wall, and extrinsic neurons (sensory afferents and sympathetic) located outside of the GI tract. Extrinsic sensory afferents are the crucial conduit of the gut–brain axis, enabling detection of a wide range of GI stimuli.[9] These afferents relay sensory information to central circuits via pathways to the spinal cord, brainstem, and brain. At the extreme, these afferent pathways drive symptoms such as nausea, vomiting, bloating, discomfort, and pain, especially when the GI tract is hypersensitive. Abnormal processing within central nervous system (CNS) circuits can amplify sensory signals, contributing to chronic pain, anxiety, and depression.[10] There are important sex-based differences in the mechanisms underlying chronic visceral pain, including its epigenetic regulation after stress.[11]

**Visceral afferent pathways.** Extrinsic sensory afferents span the entire length of the GI tract and can be classified based on the location of their cell bodies and the anatomical pathway they run through (eg, vagal or spinal).[12] The cell bodies of vagal afferents are within the nodose ganglia and send peripheral projections that are most dense in the stomach and esophagus, gradually decrease in density through the small intestine, are sparse in the distal colon, and are absent from the rectum. Functionally, vagal afferents are involved in detecting nutrient contents, fullness, and satiety in the upper GI tract.[12] Spinal afferents have cell bodies in the dorsal root ganglia (DRG) and can be further subdivided (eg, splanchnic or pelvic) based on the location of their cell bodies within specific DRG regions (eg, thoracolumbar or lumbosacral). Spinal afferents typically respond to higher-threshold, higher-intensity stimuli and signal bloating, discomfort, cramping, urgency, and pain.[12]

**Visceral afferent subtypes.** Anatomically, GI afferents have peripheral nerve endings that terminate in all layers of the GI tract and have diverse specialized structures, including endings on blood vessels, simple- and complex-type endings at crypts, branching endings in the submucosa, intraganglionic laminar endings, and intramuscular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Genetically encoded reporters
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Chromatin
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Molecular evaluation
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Artifical Intelligence (AI)
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arrays.[12] Functionally, vagal afferents conduct information via A-delta (thinly myelinated, >3 m/s) and C (unmyelinated, ~1 m/s) fibers, whereas spinal afferents are generally C fibers. Functionally, GI afferents fall into 5 broad classes: (1) mucosal (exquisitely sensitive to mucosal distortion, but not stretch/distension), (2) muscular (respond to low threshold stretch/distension), (3) muscular/mucosal (respond to mucosal distortion and stretch/distension), (4) vascular (high-threshold stretch/distension endings in the mesentery and submucosa), and (5) silent afferents (in normal conditions there are various types of chemically sensitive but mechanically insensitive afferents).[12]

GI afferents express a wide range of ion channels and receptors that govern their excitability and sensitivity. Molecular atlases of vagal afferents reveal molecular specialization, with unique mechanosensory and chemosensory afferents innervating the stomach and intestine.[13] Single-cell transcriptomics of colon-innervating afferents reveal 7 major classes, with 5 shared across splanchnic and pelvic pathways, and with 2 classes exclusive to the pelvic pathway.[14]

Elegant studies have recently demonstrated the molecular identity of vagal intraganglionic laminar endings, as well as the vagal circuits for malabsorption prevention, feeding regulation, appetite suppression, gut-induced reward, fluid osmolarity sensing, and sugar preference. Furthermore, spinal colonic afferents have been broadly molecularly subdivided into being responsive to low (tyrosine hydroxylase expressing) or high (Bmpr1b, Sstr2 expressing) distension.[15]

**Mechanotransduction.** To respond to mechanical stimuli, GI afferents rely on mechanotransduction, the conversion of mechanical force into electrical signals. This extremely rapid process involves mechanosensitive ion channels that open in response to membrane deformation, allowing ion influx and formation of generator potentials proportional to the stimulus intensity. In excitable cells, this process leads to action potential firing via activation of voltage-gated sodium ($Na_v$) and calcium channels. Although several ion channels have been identified as being mechanosensitive, the evolutionary conserved and bona fide mechanosensor, Piezo-type mechanosensitive ion channel component 2 (PIEZO2) has been identified in enterochromaffin cells and in extrinsic sensory afferents and contributes to their function. For example, PIEZO2 helps spinal afferents regulate GI transit by slowing food transit rates in the stomach, small intestine, and colon.[16]

**Chemotransduction.** An enormous range of chemical mediators influence the sensitivity of GI afferents. These mediators originate from a variety of sources, including the substances we consume, the intestinal microbiome, epithelial cells, glia, and immune cells, plus enteric and extrinsic neurons. Major mediators include proteases, histamine, adenosine 5'-triphosphate, and serotonin (5-hydroxytryptamine [5-HT]). These mediators produce their effects on GI afferents by 3 main mechanisms: (1) direct activation via ion channels or receptors on nerve terminals; (2) sensitization by increased afferent excitability, or longer-term by (3) phenotypic modulation via altering the expression profile of ion channels and receptors.[9] These changes, individually or collectively, can promote pronociceptive or antinociceptive effects on GI afferents. Key pronociceptive mechanisms include activation of (1) transient receptor potential (TRP) channels, including TRPV1, TRPV4, and TRPA1, (2) $Na_v$ channels, $Na_V$ 1.1 and $Na_v$ 1.6–1.9, (3) ionotropic receptors, including $P2_X$ and $5\text{-}HT_3$, and (4) excitatory G-protein–coupled receptors (GPCRs), including MrgprA3, MrgprC11, and MrgprD.[9] Conversely, activation of inhibitory GPCRs, such as γ-aminobutyric acid (GABA) subtype A or B receptors ($GABA_AR$ or $GABA_BR$), results in a reduction in afferent action potential firing and antinociceptive actions.[9]

**Microglia and microglia-like cells.** Enteric microglia also contribute to the control of abdominal pain, because deleting connexin-43 in these cells protects against the development of visceral hypersensitivity in preclinical colitis models.[17] Microglia-like cells (satellite glia and microglial-like macrophages) within the DRG or the spinal cord can directly sensitize GI afferent neurons to drive abdominal pain. For example, granulocyte colony-stimulating factor activates microglia-like cells via cathepsin S-CX3C motif chemokine receptor 1–inducible nitric oxide synthase signaling, while inhibiting TRPV1

**Figure 1.** Populations, tools, and techniques that contribute to the fundamental neurogastroenterology and motility knowledge. (*A*) Individuals and populations reveal the genetic and epigenetic mechanisms involved in DGBI, and animal models (nonhuman primates, rodents, zebrafish, fruit flies, hydra, and *Caenorhabditis elegans*) provide reductionist platforms to examine the physiology and genotype–phenotype connections. (*B*) Broad assessments of organ function in DGBI include measurements of brain–gut communication by imaging or biomarkers, transit of contents through the gut, and sensory function during distension of balloons to test visceral sensitivity. (*C*) Studies focusing on tissue function use organ bath approaches, such as the Ussing chamber system and muscle bath, dissociation into single-cell elements that are studied using imaging and electrophysiology, and internally using next-generation sequencing approaches to determine the genome, exome, and epigenome, as well as the transcription–translation dynamics by combining proteomics with transcriptomics. (*D*) Large data sets generated by digital approaches and next-generation sequencing studies can increasingly be interpreted using artificial intelligence (AI) and next-generation statistical approaches that cluster common features. (*E*) Functional studies are benefiting from the emergent X-genetics approaches that allow integration of opto-, chemo-, magneto-, and sonogenetics to stimulate specific cell populations and genetically encoded reporters, such as for calcium (genetically encoded calcium indicators) or voltage (genetically encoded voltage indicators), allow investigation of cellular function of specific populations. SMC, smooth muscle cell. (*F*) A range of techniques are necessary for structural investigations of the GI tract wall, which is expansive in terms of space, but which also has structural aspects, such as synapses, that require high resolution. Traditional immunohistochemistry is being supplemented by emerging techniques that allow high spatial coverage in concert with spatial resolution, using serial section electron microscopy (EM), tissue clearing, and expansion microscopy.

expressing GI afferents can prevent activation of these cells and visceral hypersensitivity in preclinical colitis models.

**Central pathways of vagal and spinal afferents.** Vagal afferents have cell bodies within the nodose ganglia and central terminals projecting into the nucleus tractus solitarius (NTS) as well as the nearby area postrema. The parabrachial nucleus is a major relay for vagal input from the NTS. It receives dense projections from the NTS and sends outputs to NTS-innervated regions (eg, hypothalamus, periaqueductal gray, and central nucleus of the amygdala [CeA]) and additional areas such as the ventroposterior thalamus, which projects to the insular cortex.[12] Spinal afferents have cell bodies in DRG, with central terminals projecting into specific laminae of the superficial dorsal horn where they synapse with second-order neurons in the spinal cord. Spinal afferents enter the spinal cord mainly via dorsal roots and run rostrocaudally along the spinal cord. Their collaterals terminate mainly in laminae I, II, and V. Dorsal horn neurons project to the brain via contralateral ascending pathways, including via the spinothalamic, spinohypothalamic, and spinoreticular tracts.[12]

**Central sensitization.** Central sensitization refers to changes in neural function within the spinal cord and brain. In the dorsal horn, 2 main mechanisms increase pain signaling: (1) enhanced ascending excitatory transmission, whereby afferents release glutamate to activate second-order neurons, amplifying nociceptive signals; and (2) reduced inhibitory control (or disinhibition), whereby inhibition of GABAergic and glycinergic interneurons lowers the nociceptive threshold. In the brain, sensitization commonly involves neuroplastic changes, particularly in the thalamus, amygdala, and insula. Preclinical models show chronic stress increases DNA methylation of the glucocorticoid receptor (GR) promoter in the CeA contributing to stress-induced visceral pain, particularly in females. Conversely, enriched environments can prevent these epigenetic changes by modulating microglial activity and altering GR and corticotrophin-releasing hormone (CRH) expression in the CeA.[18]

## Enteric Nervous System Neurobiology and Neuroplasticity

The ENS controls moment-to-moment functions of the gut by integrating signals from multiple cell types within the gut wall as well as from the brain via autonomic nerves. These roles are conducted by neurons and glia in the submucosal and myenteric plexuses that control fluid exchange across the intestinal epithelium and motor control of the smooth muscle, respectively. Defects in the ENS are likely to play a central role in DGBI and often manifest as changes in motor function, such as those observed in irritable bowel syndrome (IBS) and chronic constipation. Likewise, secretion, which may be elevated in IBS with diarrhea and decreased in IBS with constipation, is regulated, in part, by enteric neurons.

Enteric neurons are divided into 4 main functional classes: (1) intrinsic primary afferent (sensory) neurons (IPANs), (2) motor neurons, (3) interneurons, and (4) intestinofugal neurons, which are further subdivided based on specialized functions, projection patterns, electrophysiological characteristics, neurochemistry, and genomic signatures.[19] The basic arrangement of neuron subtypes and their roles are generally consistent along the digestive tract.

IPANs are in myenteric and submucosal ganglia and are the only class of enteric neurons to project to the mucosa. They are activated by specialized epithelial sensory cells, called enteroendocrine cells, which release a variety of transmitters, including 5-HT. In response to chemical and mechanical stimuli, IPANs transmit excitatory signals to multiple neurons within the plexuses. Interneurons, unipolar relay cells found in myenteric but not submucosal ganglia, are among the cells activated by IPANs. Ascending interneurons project orally and primarily use acetylcholine (ACh) and tachykinins as transmitters, whereas descending interneurons project anally and use more diverse combinations of ACh, nitric oxide, vasoactive intestinal peptide (VIP), and other transmitters. Interneurons synapse on motor neurons, which exert excitatory or inhibitory effects on longitudinal and circular smooth muscle, glands, and blood vessels.

Excitatory motor neurons are primarily cholinergic and tachykinergic, whereas inhibitory motor neurons are typically nitrergic but also release a variety of cotransmitters. Secretomotor/vasodilator neurons are rare in the myenteric plexus but represent a major portion of the neurons found in submucosal ganglia. Intestinofugal neurons have cell bodies in the myenteric plexus and project to prevertebral ganglia, where they release a variety of transmitters that influence postganglionic sympathetic neurons.

More than half of the ENS is composed of nonneuronal cells called enteric glia that regulate homeostasis within the ENS, support neuronal functions and survival, and fine-tune neurotransmission.[20] Enteric glia are diverse but can be divided into 2 main classes: “canonical” enteric glia that reside with neurons in ganglia and share transcriptional similarities with astrocytes and extraganglionic “Schwann-like” cells associated with nerve fibers that innervate the mucosa and smooth muscle coats and exhibit gene expression patterns that overlap with oligodendrocytes.[19]

### *Ganglionic Neurotransmission*

Ganglionic transmission in the ENS mainly relies on fast, nicotinic neurotransmission.[19] Mucosal stimuli trigger a polysynaptic pathway that begins with IPANs and leads to the motor pattern referred to as peristalsis. IPANs make synaptic contacts on both ascending and descending interneurons and excite these cells by releasing ACh, tachykinins, and adenosine 5'-triphosphate. Ascending interneurons form excitatory cholinergic synapses with excitatory motor neurons that, in turn, stimulate smooth muscle contraction by releasing ACh and tachykinins. Descending interneurons form excitatory cholinergic, purinergic, and serotonergic synapses with inhibitory neurons, which produce relaxation by releasing nitric oxide, VIP, and purines. Subpopulations of glia are specialized to detect neurotransmitter release from neuron subtypes

and neural pathways.[20] Enteric glia express receptors for excitatory neurotransmitters that couple to excitatory signal transduction cascades and intracellular calcium responses. These promote gliotransmitter release through the opening connexin-43 hemichannels, which lead to the release of transmitters such as adenosine 5'-triphosphate and prostaglandin $E_2$ that act on receptors expressed by the adjacent enteric neurons to modify their activity.

### *Neuroeffector Transmission*

Neuromuscular transmission to the circular and longitudinal smooth muscle involves both excitatory and inhibitory mechanisms acting on a syncytium of interstitial cells of Cajal (ICCs) platelet-derived growth factor receptor-$\alpha$–positive (PDGFR$\alpha^+$) fibroblast-like cells, and smooth muscle cells.[21] Nerve terminals predominantly innervate ICCs and PDGFR-$\alpha^+$ cells, which are coupled to each other and to smooth muscle via gap junctions. Intestinal smooth muscle is maintained in a state of tonic neurogenic inhibition by nitrergic motor neurons. During propulsive motility, oral contraction and aboral relaxation generates a pressure gradient that propels the luminal contents along the intestine. The inhibitory component of neuromuscular transmission is driven by nitric oxide and purine release, whereas the excitatory component is driven by ACh release. When acted upon by nitric oxide and purines, the syncytium made up of smooth muscle, ICC, and PDGFR-$\alpha^+$ produces tonic hyperpolarization of intestinal smooth muscle cells. In contrast, ACh release activates muscarinic receptors expressed by ICCs and smooth muscle to cause depolarization.

Neuroepithelial transmission by enteric neurons regulates water and electrolyte balance.[19] Normal fluid and mucus secretion lubricates the bowel, facilitates motility, and aids digestion, whereas excess fluid secretion is a protective mechanism that helps to flush out noxious substances. Mucosal stimuli trigger secretomotor responses by stimulating IPANs directly or through epithelial signaling, which in turn, activate myenteric interneurons and secretomotor neurons. Secretomotor neurons send projections to the epithelial crypts where they release ACh and VIP to stimulate muscarinic and VIP receptors expressed by chloride-secreting crypt epithelial cells.

### *Enteric Neuroplasticity*

Acute or chronic GI inflammation has numerous long-lasting effects on the ENS that are relevant for understanding the etiology of DGBI.[22] Changes induced by inflammation are evident at the level of single neurons and their synaptic connections in the ENS. Some have proposed that the effects of inflammation are imprinted in the ENS as a form of long-term memory.[23] Inflammation-induced changes occur in both myenteric and submucosal plexuses. IPAN hyperexcitability is prominent feature after acute inflammation and is driven through mechanisms that involve effects of prostaglandin $E_2$ and cyclooxygenase-2. Synaptic communication between neurons also increases after inflammation in part, through mechanisms that increase the readily releasable pool of synaptic vesicles and change presynaptic terminal neurochemistry. These changes persist for weeks after an acute inflammatory event in rodents and manifest as increases in both fast and slow excitatory synaptic inputs to neurons.

Extensive neurodegeneration, driven through mechanisms involving purinergic receptors, contributes to changes that occur in cellular activity and synaptic connectivity after intestinal inflammation. Neuronal adenosine 5'-triphosphate release through pannexin-1 channels stimulates the surrounding enteric glia, which subsequently drive neuron death. Enteric glia exhibit profound changes in response to inflammation that affect both neuron function and survival. Enteric glia adopt a “reactive” phenotype in response to pathophysiological perturbations that involves a complex, disease- and context-dependent continuum of dynamic states, which may be beneficial or detrimental depending on the context.[20] There is some regenerative potential in the ENS after an inflammatory or infectious insult.

Changes in the enteric neurocircuitry induced by inflammation produce long-lasting changes in GI motility and secretory function.[22] Neuronally mediated colonic secretory and motor functions follow the time course of changes in synaptic transmission, neuronal excitability, and neuronal survival and remain impaired for weeks after colitis in experimental animals. Changes in motor function are amenable to experimental therapies aimed at normalizing ENS function, limiting neuron death, or protecting against the decline in neuromuscular transmission.

## Neuroimmune Function

The GI tract contains ~75% of the body’s immune cells and a vast ENS comprising ~100 million neurons. The ENS and CNS communicate extensively via ~100,000 extrinsic nerve endings and humoral signaling pathways. The immune system profoundly influences the development and function of the GI tract, and ongoing neuroimmune interactions throughout life guide the normal and pathologic functions of both the nervous and immune systems. These interactions are important contributors to DGBI and recovery from acute insults.

Interestingly, the immune system receives foundational education within the gut, priming it for systemic functions even before birth, with further refinement occurring in secondary immune organs such as the thymus. Intriguingly, some gut–immune signaling mechanisms remain intrinsic to the gut wall and leave no systemic fingerprints, emphasizing the complexity of localized communication.[24] Neuroimmune interactions critically depend on luminal contents, including nutrients and microbial metabolites. Adding further complexity, these interactions in the gut may be influenced by signaling originating in remote organs, such as the liver and spleen, and CNS. Collectively, the multiple layers of interaction represent a dynamic and multifaceted network essential to understanding GI homeostasis and disease.

Neurons and immune cells communicate through indirect paracrine signaling and direct neuroimmune synapses,

forming a vibrant cross-talk platform and leading to an emerging concept of stable neuroimmune cell units (NICUs).[25] Immune cells express receptors for classic neurotransmitters, whereas neurons carry receptors for immune-derived signaling molecules such as cytokines. For example, nearly all leukocytes express functional adrenergic receptors, and macrophages possess ACh receptors, a major neurotransmitter in enteric signaling. Additionally, immune cells themselves can produce neurotransmitters like ACh, complicating assumptions about the origin of signaling molecules in the gut.[26] Conversely, sensory neurons express receptors for a wide array of immune signals, including cytokines and lipid-derived mediators such as prostaglandins, histamine, and growth factors. The capacity of sensory neurons to detect immune-derived signals enables them to localize and mitigate infectious challenges both locally in the gut and at systemic levels.

Immune signals drive neuronal activity through multiple pathways. First, neurons express diverse cytokine receptors that sensitize them and, under specific conditions, can even drive cytokine-specific action potentials, allowing immune signals to elicit varied neural responses.[9] Single-cell omics are aiding in mapping these vast communication channels through connecting ligand-receptor pairs across neuronal and immune populations.

Second, cytokines and other immune signals can sensitize previously silent sensory neurons or modulate their firing thresholds, altering the neuronal responses to stimuli. For instance, interleukin (IL)-1$\beta$ modifies the biophysical properties of $Na_v$, changing their neuronal firing patterns. Macrophages may even directly regulate neuronal electrical activity, highlighting an especially close neuron–immune cell interface. Finally, microbial metabolites, acting locally and remotely, play essential roles in neuroimmune communication and represent expanding potential therapeutic targets.

Interactions between neurons and immune cells might be more stable and integrated than previously thought, supporting the NICU concept. In addition to immune cells and neurons, several other cell types, such as enteroendocrine, glial, smooth muscle, and interstitial cells, as well as microbiota, actively shape neuroimmune signaling by producing or modifying signaling molecules such as catecholamines.[27]

The ENS primarily regulates local functions, and its functions are shaped by extrinsic inputs, including sympathetic, parasympathetic, and sensory neurons. Next-generation sequencing has elucidated significant diversity within enteric neuronal populations, identifying functional subclasses, such as possible multiple classes of IPANs, and uncovering novel neuron classes involved explicitly in neuroimmune interactions. A notable example includes reciprocal communication between macrophages and neurons mediated by signaling molecules such as macrophage-derived bone morphogenetic protein 2 and neuron-derived colony stimulating factor 1, which modulate each other's functions, and both are fine-tuned by the gut microbiome.[28]

Gut-projecting extrinsic afferents expressing TRPV1 and $Na_V1.8$ provide another critical link between the immune and nervous systems. These neurons, innervating Peyer's patches and other mucosal immune structures, collaborate with epithelial and mucosa-associated immune cells via calcitonin gene-related peptide and substance P signaling, contributing significantly to gut defense against pathogens like *Salmonella typhimurium*. Sympathetic and parasympathetic neurons, using neurotransmitters such as norepinephrine and ACh, respectively, engage directly with tissue-resident macrophages to shape their phenotype and functional states. Vagal nerve stimulation triggers cholinergic anti-inflammatory pathways involving direct communication with macrophages through $\alpha$7 nicotinic receptors. Such intricate neuron-immune cross talk underscores the fundamental importance of extrinsic neural input in immune regulation.

The immune system's classical division into innate and adaptive branches encompasses cells that interact closely with neurons to regulate GI physiology. Innate immune responses, characterized by rapid, nonspecific inflammation via pattern recognition receptors, involve granulocytes (neutrophils, eosinophils, basophils, and mast cells), macrophages, dendritic cells, and innate lymphoid cells (ILCs). Certain lymphocytes, such as $\gamma\delta$T cells and natural killer T cells, bridge innate and adaptive functions. Granulocytes densely populate the gut mucosa, contributing variably understood roles in neuroimmune interactions.

Mast cells, which have garnered the most interest, frequently associate closely with enteric neurons, increasing their interactions during inflammatory states, such as in IBS or helminth infections. Mast cell activation modulates neuronal activity, either sensitizing neuronal receptors or limiting excitability through neurotransmitter degradation, highlighting bidirectional regulation within NICUs.

Macrophages, the gut's most abundant immune cells, exhibit significant diversity related to their origin, localization, and functional specialization. Resident embryonically seeded macrophages coexist alongside monocyte-derived macrophage populations. These macrophages interact closely with neurons across distinct gut niches, with functional phenotypes varying from proinflammatory in lamina propria to anti-inflammatory in the muscularis.[29] Although not technically part of the ENS, muscularis macrophages reside at the level of the myenteric plexus and interact with multiple cell types including neurons, glia, ICCs, PDGFR-$\alpha^+$ fibroblast-like cells and smooth muscle cells. Muscularis macrophages fill a functional niche like microglia in the brain and exhibit bidirectional interactions with enteric neurons that have reciprocal effects on cellular function and survival. Neuronal–macrophage interactions influence neuronal development, maintenance, and function, reciprocally shaping macrophage phenotypes in infection and inflammation.

ILCs[30] encompassing the subsets, ILC1, ILC2, and ILC3, and natural killer cells, regulate immune responses and barrier integrity via cytokines. ILC2 cells coordinate anti-helminth responses and tissue repair through IL5 and IL13 secretion, modulated by neuronal signals such as neuromedin U and sympathetic $\beta_2$-adrenergic signaling. ILC3

cells interact closely with VIP-expressing enteric neurons to balance lipid absorption with immune homeostasis, simultaneously influencing CNS-associated neuroinflammation. Finally, neuronal activities within ENS and CNS profoundly shape immune functions in the GI tract.

Circadian rhythms regulate intestinal immune responses and microbiota composition through modulation of ILC3 populations. Vagal afferent sensing of microbiome-derived signals via the liver impacts CNS integration, subsequently influencing colonic T-cell populations through vagal efferents and local enteric neurons. Sympathetic and sensory neuronal innervation of GI tract–associated lymphoid tissues modulates epithelial and immune responses to pathogens, regulating pro- and anti-inflammatory processes through receptor-specific mechanisms.[31]

Collectively, the intricate neuroimmune interactions within the GI tract encompass diverse cell types, signaling molecules, and pathways. The remarkable complexity, facilitated by transformative single-cell and spatial transcriptomic technologies, continues to reveal profound insights into GI physiology, offering novel therapeutic avenues for GI disorders driven by neuroimmune dysregulation.

## The Role of the Microbiome in Gut–Brain Interactions

Host–microbe interactions are facilitated by the framework of the gut–brain axis, allowing a microbial influence on multiple fundamental aspects of host physiology, including GI and brain function and behavior. The gut microbiota is also capable of exerting direct and indirect impacts on host metabolic pathways to regulate the supply of neuroactive molecules and their precursors to both levels of the gut–brain axis (Figure 2).

Gut microbiota–ENS interactions dictate a range of GI functions, including an influence on GI musculature, secretory glands, and blood vasculature via microbial-derived components or microbial metabolites, possibly via ion channels. Pattern recognition receptors expressed in the ENS provide another possible recognition mechanism for microbial molecules. The aryl hydrocarbon receptor, expressed on enteric neurons, also detects microbial signals from the intestinal lumen to influence gut physiology.[19] The plasticity of the adult ENS and region-specific postnatal development of the ENS may also be contingent on early exposure to the gut microbiota. ENS development also occurs in parallel with the assembly trajectory of the gut microbiota and the priming and education of the immune system.[32] Triangulating these conversations at the interface of the luminal ecosystem and beyond can be aided by the application of single-cell transcriptomics, circuit-tracing methods, and functional chemogenetics to pinpoint gut microbiota contributions to the normal functional activity of the ENS, as well as its integration into gut–brain axis neurocircuitry.

Microbiome gut–brain interactions are supported by converging evidence from a variety of experimental strategies implicating the gut microbiome in stress physiology as well as behaviors relevant to anxiety, depression, visceral pain, cognition, and social interactions. Alterations have been detected in the hub brain regions involved in the neurocircuitry underpinning these behaviors and implicating specific associated mechanisms, including hippocampal neurogenesis, serotonergic neurotransmission, neuronal function, myelination in the prefrontal cortex, and hypertrophy of neurons of the basolateral amygdala.

These observations are now supported by studies in relevant clinical populations, highlighting compositional and functional alterations in the gut microbiota, including in depression, autism, and schizophrenia. Systematic reviews and meta-analyses report a transdiagnostic pattern of microbiota signatures in depression, bipolar disorder, schizophrenia, and anxiety, including a reduced relative abundance of specific bacterial taxa and genera that produce short-chain fatty acids (SCFAs) and a higher relative abundance of lactic acid–producing bacteria and bacteria associated with glutamate and GABA metabolism.[33] Establishing a causal role for disease-associated microbiota configurations currently relies on fecal microbiota transplantation, most frequently from human donors to recipient animals. This has, for example, been demonstrated in the case of depression, visceral pain, schizophrenia, and comorbid GI motility and anxiety in IBS.[34]

The functional implications associated with the role of the vagus nerve in host–microbe interactions extend across mood and emotion, reward, cognitive function, digestive function, energy homeostasis, and immune responses. The pathways activated in the CNS arising from local GI microbe–vagus nerve interactions reflect key projection sites of gut-related vagal afferents in the brain and include alterations in central GABA receptor expression and hippocampal neurogenesis. More recent developments include a neural gut–brain circuit for reward, a circuit linking GI-derived vagal sensory signaling to hippocampal-dependent memory function, and “neuropod” enteroendocrine cells that facilitate a neuroepithelial relay circuit that connects the intestinal lumen to the brainstem.[32]

The gut microbiota is critical in the development of the immune system, and the composition and function of the gut microbiome is also heavily influenced by the immune system. Bacterial polysaccharides and peptidoglycans are important examples of conserved microbial molecular signatures recognized by host pattern-recognition receptors. Other immunomodulatory microbial metabolites include bacteriocins, bile acids, and SCFAs. Low-grade immune activation is a hallmark of stress-related disorders, such as depression and IBS, and this may originate at GI mucosal sites before spreading to distal organs. Links between the gut microbiota and neuroinflammation have come under much scrutiny in the context of maturation and function of microglia in the CNS, for example, and neuroinflammation is associated with microbial metabolites such as the SCFA, acetate, and indoles produced from tryptophan, linked to downstream neurogenesis and functionally associated with learning and memory. SCFAs have also been identified as proregenerative modulators of poststroke neuronal

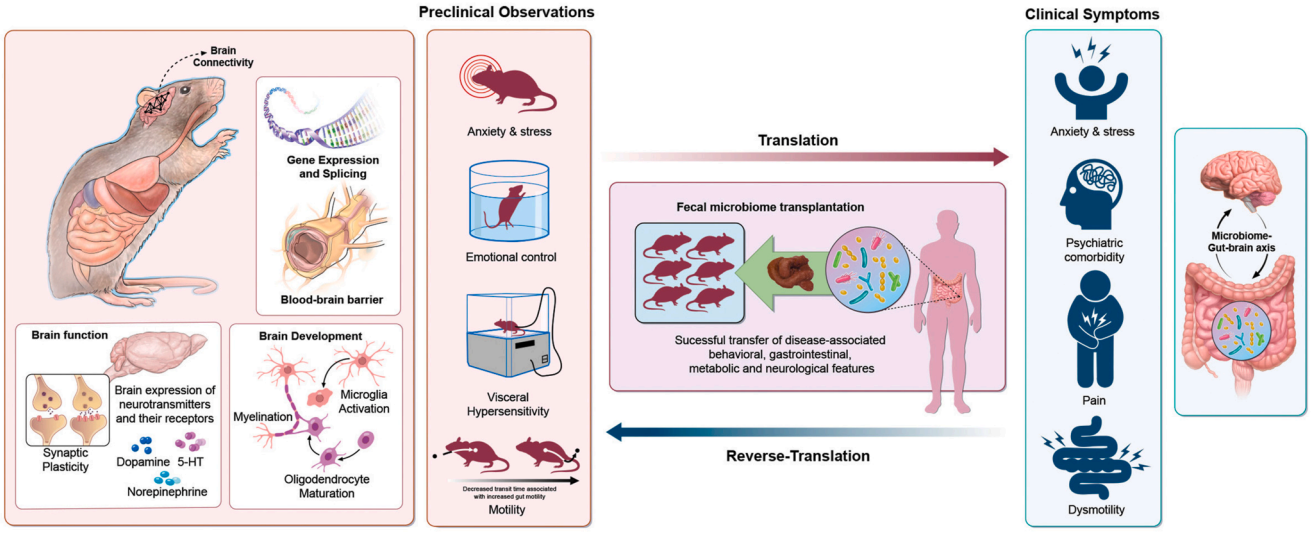


**Figure 2.** Preclinical research has revealed how the gut microbiota impacts multiple behavioral and molecular features relevant to DGBI. This includes profiles related to depression, anxiety, and pain as well as dysmotility. There is evidence, for example, that the visceral hypersensitivity that underpins abdominal pain is regulated by the gut microbiota, including the recruiting of CNS sites involved in pain processing. These observations have prompted translational efforts to evaluate gut microbiota composition and function in relevant clinical disorders, revealing associations between key GI and psychological symptom profiles. Fecal microbiota transplantation studies have been deployed in reverse translational approaches and have indicated a potential causal role for disease-associated gut microbiota configurations in recapitulating the core symptoms in recipient animals.

plasticity in an experimental stroke model. Neuroinflammation can arise from the expansion of segmented filamentous bacteria in the gut or from the modulation of splenic Ly6C$^{high}$ monocyte function, innate immune cells implicated in the development of CNS-related disorders.[35]

Microbial regulation of neuroendocrine pathways involves the hypothalamic–pituitary–adrenal (HPA) axis, a key pillar of the gut–brain axis with an important role for the gut microbiota in the postnatal priming and development of the HPA stress response. Acute and chronic stress exposures can also impact the composition and function of the gut microbiota. Neuroendocrine pathways are associated with addiction, learning, and memory, sexual behavior, eating behavior, and social interactions. Outside of glucocorticoids, other hormones secreted by enteroendocrine cells, such as cholecystokinin, peptide YY, and glucagon-like peptide-1, have also been implicated. Another important neuropeptide influenced by the gut microbiome in this context is oxytocin, which is implicated in social behavior, addiction, learning and memory.[32]

Microbial metabolic pathways are important considerations in gut–brain axis signaling. Important examples include SCFAs, bile acids, indole metabolites produced from tryptophan, and neurotransmitters. Microbial degradation of testosterone with implications for depression has also been reported. Microbial metabolic pathways linked to neuroactive compound production or degradation processes (eg, GABA production, tryptophan metabolism) have also been catalogued as gut–brain modules. Bacteria-derived tryptamine, acting as a ligand for the gut epithelium–expressed 5-HT$_4$ receptor to control gastrointestinal motility, is one example of a microbial metabolite that can interact with host GPCR receptors to control physiology.[32] Gram-negative bacteria produce microbial microvesicles or extracellular vesicles that are capable of influencing gut physiology, brain function, and behavior via the action of their cargo on the peripheral nervous system and immunomodulation. There is currently much interest in determining whether these microvesicles can cross the blood–brain barrier with implications for neuroinflammation and neurodegeneration.[36]

## Enteric Nervous System-Central Nervous System Abnormalities in Disorders of Gut–Brain Interaction

### *Relationship With Psychiatric Comorbidities*

Up to half of individuals diagnosed with a DGBI also present with a psychiatric comorbidity, most commonly anxiety. Even those not diagnosed formally with a psychiatric condition, patients possess excessive symptom-specific fear and distress.[37] There is substantial evidence in humans and animal models that psychological stressors play important roles in the onset and perpetuation of IBS symptoms, suggesting that stress alters visceral nociception and may play a role in central pain amplification and worsened disorder severity.[11]

### *Alterations in the Hypothalamic–Pituitary–Adrenal Axis in Inflammatory Bowel Syndrome*

An underlying connection between anxiety and DGBI is the HPA axis, a major stress pathway in mammals. The

human networks that link anxiety and autonomic nervous system output with gut sensitivity, permeability, and motility share significant homology to a stress-induced visceral hyperalgesia circuit in rodents that implicates the involvement of CRH in the regulation of brain–gut interactions in IBS. Under homeostatic conditions, HPA activation initiates a negative feedback loop with resultant inhibition of CRH production.[11,37] In contrast, when cortisol binds to the CeA instead, CRH expression is promoted with a resultant augmentation of stress axis activation. Acting as a node that integrates emotional, sensory, and visceral information, the CeA thus links stress and anxiety to subsequent alterations in GI in motility, permeability, and nociception.[11] Accordingly, clinical studies have confirmed a role for HPA hyperactivation, hypercortisolemia, and dysregulated colonic motility responses to exogenous CRH in IBS patients. Of note, CRH receptors and CRH are also located in the GI tract where they play a more direct role in GI function.

### *Psychiatric Comorbidities Alter Specific Signaling Pathways to Induce Inflammatory Bowel Syndrome Phenotypes*

Among the neurotransmitters and neuromodulators that underlie GI reactions to stress, the 5-HT and endocannabinoid-vanilloid systems are among the most highly studied. 5-HT is a key modulator of ENS and CNS development, mood, and DGBI-related symptoms, including GI motility, visceral nociception, and secretion.[38] 5-HT initiates these actions via activation of multiple 5-HT receptors. Most DGBI-related research has focused on 5-$HT_3$ and 5-$HT_4$ (for more information, see the 5-HT section below).[38]

Pathways mediated by cannabinoid-1 ($CB_1$) and TRPV1 receptor have been demonstrated to link visceral hypersensitivity to chronic and early-life stress (ELS) through HPA axis activation, altered expression of $CB_1$ and TRPV1 receptors in the DRG, or induction of epigenetic changes, or both. Targeting these pathways may be quite complex, however, because $CB_1$ and TRPV1 receptor activation has been shown to elicit opposing actions, and how this modulation occurs is uncertain.[39]

### *Descending Supraspinal Structures Innervating the Gut and Their Activation by Psychiatric Comorbidities and Stress*

Imaging studies have revealed patterns of central neural activation in IBS patients that include elevated activity in sensorimotor, emotional arousal, and salience networks in the amygdala, insula, cingulate, and prefrontal cortex. These CNS regions are involved in fear, learning, and anxiety, as well as changes in gut motility and nociceptive responsiveness to colorectal distention.[38] In rodent models, elevation of CeA corticosterone levels by stereotaxically implanted corticosterone micropellets increases sensitivity to visceral and somatic stimuli and induces anxiety-like behavior.[11] Activation of descending facilitatory pathways is also reported to be involved in the exacerbation of IBS symptoms in response to stress, whereby their activation induces remodeling to visceral pain via sensitization of spinal dorsal horn neurons.

The brainstem also plays key roles in perpetuating pain and stress. The periaqueductal gray receives excitatory and inhibitory signaling from the prefrontal cortex and amygdala, respectively, whereas the rostroventral medulla receives nociceptive information from the spinoreticular pathway as well as integrated pain and stress signals from amygdala and periaqueductal gray responses.[11,37] Opioids, 5-HT, and noradrenaline signaling, among others, are thought to underlie the analgesic actions of supraspinal pathways.

### *Stress Induces Epigenetic Changes Leading to Inflammatory Bowel Syndrome Phenotypes*

Remodeling of the epigenome during chronic stress may result in long-term changes in gene expression within the limbic system that have been linked to the development of visceral nociception. In both an adult murine model of stress induced by water avoidance and in ELS, CRH promoter hypomethylation and an increase in CRH expression or CRH transcriptional responses to stress in adulthood, respectively, ensued.[11,37] Conversely, direct CNS administration of a histone deacetylase inhibitor reverses visceral hypersensitivity induced by repeated water avoidance stress or activation of the CeA with corticosterone. Other aspects of the HPA axis are also affected by stress, as shown in clinical studies; the GR gene in offspring is increased by parental psychopathology and early-life trauma. Antenatal stress, from maternal experience of war or domestic violence, affects the epigenetic profiles of GR and GR-related genes in blood samples from newborn and adolescent progeny.[11,37]

### *Stress and Neuroimmune Interactions Leading to Inflammatory Bowel Syndrome Pathophysiology*

Stress concomitantly increases innate and adaptive immune activation, gut permeability, and visceral hypersensitivity.[40] Animal studies show that acute stress increases mast cell numbers, mast cell degranulation, and gut permeability, and one of the major ways that CRH has been shown to exert its functions is via mast cell activation. In IBS, mast cell abundance is correlated with clinical symptoms, including abdominal pain and bloating.[41] Mast cell numbers are increased in colonic biopsy specimens of IBS patients (vs noninflamed controls), and colonic mucosal mast cells are activated by CRH to secrete their mediators, including histamine, proteases, and cytokines, that activate sensory neurons isolated from the DRG or alter intestinal muscle contraction, or both. Importantly, therapeutics targeted to mast cell stabilization or histamine H1 receptor antagonism successfully reduce abdominal pain in some IBS patients as well as visceral hypersensitivity in a rat model of stress-induced visceral hypersensitivity.[41] Mast cell activation also stimulates T lymphocytes to produce

inflammatory cytokines, including IL6, that has been found to be increased in the circulation of individuals with IBS.

## Risk Factors for Disorders of Gut–Brain Interaction Development

### *Basic Science of Pertinent Enteric Nervous System Developmental Pathways*

Although most ENS development occurs in the fetus, the electrophysiological properties and synaptic profile of enteric neurons are still immature in postnatal day 10 mice, which is similar in development to the full-term infant gut. In early postnatal life, the ENS undergoes continued enteric neurogenesis and gliogenesis, as well as neuronal sprouting, neurotransmitter synthesis, synapse formation, and neuronal pruning, which together shape the adult ENS phenotype. Enteric gliogenesis and enteric neuronal turnover may continue to occur throughout life, and changes in synaptic contacts within the ENS circuitry occur in mice at least through adolescence. The ENS is thus vulnerable to insults occurring throughout these periods. Furthermore, influences thought previously to be critical exclusively for prenatal development, such as rearranged during transfection, have been demonstrated postnatally and may play differing roles depending on their presence during specific stages of development.[19]

Some key perinatal factors that have been demonstrated to modulate ENS development include genetic differences, sex steroid hormones, and maternal or early postnatal exposures, including stressors, antibiotics, and diet. Gut microbiome shifts occur in response to many of these exposures and may thus be an important mechanism by which changes occur. Furthermore, alterations in transmitter-based pathways may also result from different types of prenatal or postnatal exposures, or both. For instance, dysregulation of 5-HT homeostasis may represent a common pathway originating from any or all of stress, infection, or inflammation to influence the properties of the mature ENS. The effects of environment-induced alterations in 5-HT or other signaling pathways on the ENS may also provide insight into the genesis of GI dysfunction that sometimes accompanies CNS disorders, such as links between anxiety or depression and DGBI-related symptoms in individuals.[42,43]

### *Enteric Nervous System Development and Plasticity: Genetics and Epigenetics*

A heritable component of IBS has been suggested through multiple study types. Interestingly, based on classical candidate-gene approaches, 2% to 4% of individuals with IBS have been found to possess pathogenic variants in the sucrose-isomaltase gene, possibly linking symptoms in some patients to those consistent with disaccharide intolerance. Additional associations with common single-nucleotide polymorphisms have been reported, but many studies are underpowered, negating the ability to detect modest genetic risk effects with genome-wide significance. Further research is also needed to determine whether these genetic risk variants are involved in ENS development or function, or both.

### *Enteric Nervous System Development and Plasticity: Early-Life Stressors*

Children exposed to ELS experience a 2-fold increased risk of developing IBS, yet the mechanistic links between ELS and DGBI remain poorly understood. Rodent models of ELS, such as maternal separation, limited nesting, or odor-shock conditioning, which attempt to model early childhood stressors, can produce chronic, sexually dimorphic increases in visceral sensitivity, GI dysmotility, increases in gut permeability, or a combination of these, in adulthood.[37] ELS causes dysregulation of the HPA axis, resulting in a heightened stress response that may explain the link between DGBI and trauma more broadly. Estrogens and androgens may impact sex-dependent developmental pathways through differential modulation of HPA axis activation, autonomic nervous system regulation, gut microbiota composition, or a combination of these. Which trauma-related descending pathways or transmitters generated are directly linked to changes in ENS development and function still requires exploration.

### *Microbiota and Enteric Nervous System Plasticity*

The early postnatal period of ENS and CNS development can be influenced by gut microbiota, including by the release of bioactive molecules, including gut neurotransmitters, SCFAs, or bile acids.[38] Other microbiota-mediated developmental mechanisms include activation of GPCR-mediated signaling pathways, microbial–epithelial interactions, and aryl hydrocarbon receptor regulation. The role of microbiota on ENS development and function has been most clearly delineated in gnotobiotic mice that possess a reduced number and different subtype distributions of enteric neurons, deficits in GI motility, altered enteric glial cell development, and attenuated excitability of IPANs. Conventionalization of adult germ-free mice with specific pathogen-free microbiota, probiotics, or specific bacterial strains reduces many ENS-associated deficits, implicating a role for the gut microbiota in ENS plasticity.[38]

### *Antibiotic Exposure*

Antibiotic exposure during infancy and childhood results in significant changes in CNS and ENS development, with consequent increased susceptibilities to DGBI and associated mental health conditions.[44] Most data have emerged from studies in the CNS where early-life exposure to broad-spectrum antibiotics in mice, even at subtherapeutic levels, results in findings comparable to those seen in germ-free mice, including long-lasting impairments in social behaviors, CNS neurogenesis, cognitive function, or a combination of these.[45] These changes are accompanied by alterations in neurodevelopmental gene expression pathways affecting emotional and cognitive functions in CNS regions that are vulnerable to early-life perturbations,

acutely sensitive to maternal rearing, and are shown to be dysregulated in some individuals with IBS. As in the CNS, in the ENS, mice exposed to early-life antibiotics, with resulting microbiota alterations, or germ-free mice also show abnormalities in enteric neuronal and mucosal glial numbers, neuronal reflexes, long-term dysmotility, and visceral pain, suggesting a significant impact of gut microbiota on multiple facets of ENS development and function.

### *Microbiome and Diet*

In infancy, microbiota composition largely depends on whether the diet is composed of breastmilk or formula. *Lactobacilli* and *Bifidobacteria* are present in human breast milk and are thought to exert beneficial effects on neurobehavior and cognitive development. *Bifidobacteria* species also stimulate production of SCFAs, a factor important for ENS plasticity and GI motility. The period after weaning encompasses marked shifts in the gut microbiota to a more adult-like composition that is linked to solid food introduction which may play a larger role in ENS maturation. A resistant starch diet that is known to produce SCFAs has been shown to increase the proportion of excitatory cholinergic neurons in the colon and lead to an increase in colonic transit time, suggesting that some SCFAs may induce ENS plasticity. Currently, there is little understanding of the mechanisms by which the diet impacts ENS development. Mechanisms that have been studied include activation of Toll-like receptors, microvesicles, neurotrophic factors, epigenetic changes, and other microbiota-generated metabolites.[38]

## Serotonergic Signaling as a Conceptual Nexus in Disorders of Gut–Brain Interaction Development Symptomatology and Pathophysiology and Implications for Disorders of Gut–Brain Interaction Development Treatment

The overwhelming majority of 5-HT is synthesized in the intestine where it has been shown to be important in the modulation of motility, permeability, visceral pain, and anxiety.[10,46] Although this section focuses on intestinal 5-HT, it is also produced in the CNS where it has been shown to be important in the modulation of mood disorders that frequently co-occur with DBGI[43] (Figure 3).

### *Serotonin Synthesis, Signaling, and Inactivation*

Most intestinal 5-HT is produced by the enterochromaffin cells of the epithelium, with a minority synthesized by enteric neurons via their rate-limiting enzymes, tryptophan hydroxylase 1 (TPH1) and TPH2, respectively.[46] Antagonism or loss of TPH1 thus results in depletion of 5-HT in the intestinal epithelium without continued production in the CNS and ENS. In contrast, ablation of TPH2 results in eradication of 5-HT production in the CNS and ENS with retention in the intestinal epithelium.

Once secreted, 5-HT can exert its actions via activation of 5 different receptor subclasses—5-$HT_1$, 5-$HT_2$, 5-$HT_3$, 5-$HT_4$, and 5-$HT_7$—that distribute differently throughout the GI tract.[46] 5-$HT_3$, 5-$HT_4$, and 5-$HT_7$ are present on muscle, whereas 5-$HT_{2B,}$ 5-$HT_3$, and 5-$HT_4$ are expressed on extrinsic primary afferent neurons, and 5-$HT_3$ is present on vagal afferent nerves, both of which facilitate afferent signaling.[46] The receptors that have been investigated most comprehensively as therapeutic targets for DGBI are 5-$HT_3$ and 5-$HT_4$. 5-$HT_3$ receptor antagonists slow GI motility and decrease propulsive activity by blocking 5-$HT_3$ receptors on IPANs, interneurons, and motor neurons. Furthermore, blocking 5-$HT_3$ receptors on extrinsic sensory afferents is antinociceptive.[46] 5-$HT_4$ agonists, used to treat IBS with constipation and chronic constipation, increase colonic motility and decrease visceral hypersensitivity, at least in part, by activating 5-$HT_4$ receptors on enteric neurons and intestinal epithelial cells or supraspinal neurons, respectively.[47]

5-HT must be transported intracellularly to undergo inactivation.[46] This transport is primarily conducted by the plasmalemmal serotonin reuptake transporter (SERT), that is present in intestinal enterocytes and a subpopulation of enteric neurons. SERT knockout murine models or mice that are exposed during gestation to selective serotonin reuptake inhibitors (SSRIs), display differences in in vivo and ex vivo motility and show differences in visceromotor response to colorectal distention.[42] The motility abnormalities in both SERT knockout and SSRI-exposed models can be reversed by ablation of efferent sympathetic signaling, suggesting that intestinal SERT can modulate motility through brain–gut signaling.[42] Therapeutics targeting SERT, such as SSRIs, have been used more frequently in patients with DBGI and comorbid mood disorders, although their efficacy and precise mechanism(s) of action are unclear. SSRIs have been shown to slow GI transit time in individuals with IBS with diarrhea, and some studies have shown efficacy in alleviation of abdominal pain.

## Conclusion and Future Directions

This review emphasizes the need for interdisciplinary expertise and approaches, preclinical models, and exploring genetic, epigenetic, and microbial influences to advance the understanding and treatment of DGBI. As a result of the rapid advances in several enabling technologies, neurogastroenterology will be poised to support the transformation of knowledge from the mechanism-based research setting into improved *targeted* and *individualized* clinical diagnoses and companion precision treatments for patients with DGBI. Pivotal future discoveries in the field of DGBI include understanding genetic and epigenetic analysis in cells and tissues and then translating them to organ-level physiology. Determining the importance of genes to cell–cell interactions that include neural, immune, and neuroimmune communication where there are blurred definitions will be important, especially as they relate to immune

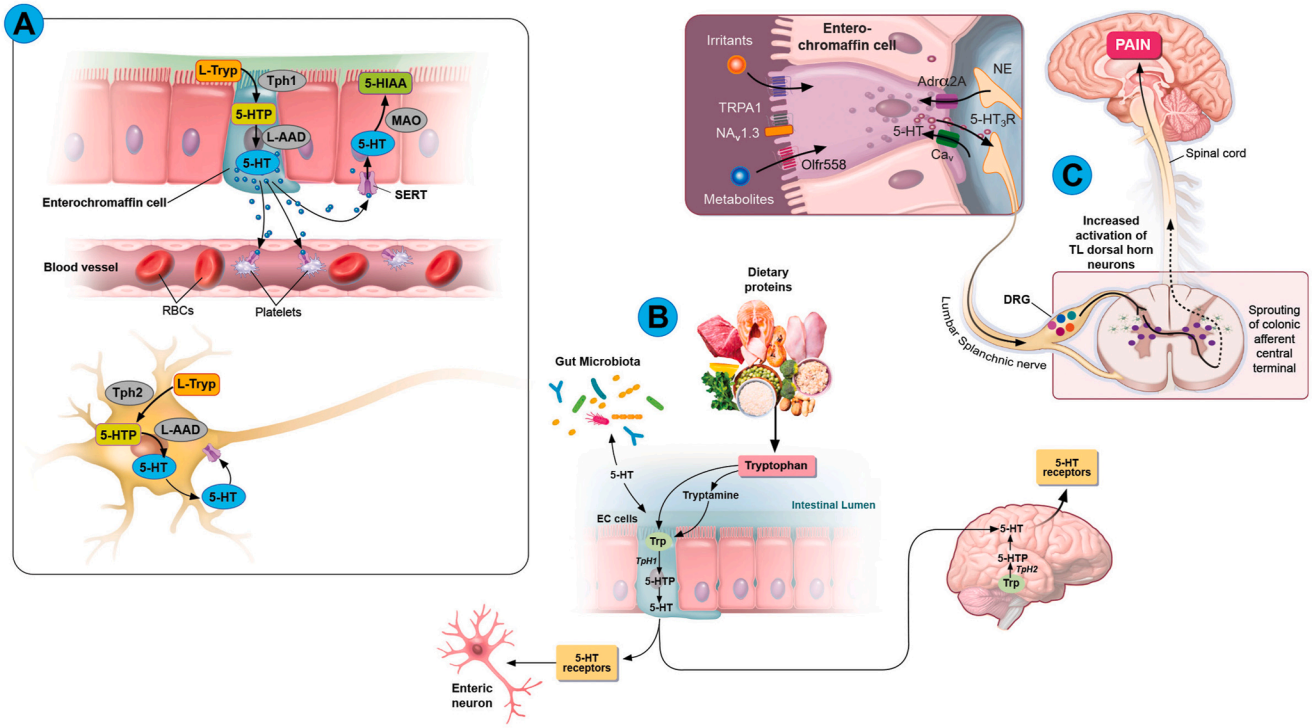


**Figure 3.** Serotonin as a nexus for DGBI. (*A*) 5-HT is produced in the GI epithelium in enterochromaffin (EC) cells by the biosynthetic enzyme TPH1. Once produced, it can be secreted basolaterally to the blood, where most is taken up by platelets that contain the SERT. 5-HT is also produced in enteric neurons by TPH2. Once released and secreted, a significant portion can also be rapidly taken up into GI epithelial cells or enteric neurons where it is inactivated in the mitochondria. L-AAD, L-amino acid decarboxylase; MAO, monoamine oxidase; RBC, red blood cell. (*B*) 5-HT can also be secreted to activate IPANs to in turn activate GI motility in enteric neurons or extrinsic primary afferent neurons (ExPANs), where it stimulates pathways in the CNS. (*C*) Stimulation of $5\text{-HT}_3$ receptors on ExPANs by 5-HT have been shown to elicit visceral pain through signaling pathways, including the DRG and spinal cord. $Ca_v$, voltage-gated calcium channel; NE, norepinephrine; TL, thoracolumbar. Tryptophan (panel *A*) is a precursor for 5-HT, and once converted to tryptamine (panel *B*), can be used by TPH1 to generate 5-hydroxytryptophan (5-HTP) and then 5-HT. Specific dietary proteins and gut microbiota (panel *B*), as well as gut irritants or metabolites (panel *C*) can elicit tryptophan production or 5-HT production and secretion, or both.

signaling and enteric neurons and considering the relative meaning of afferent signals setting immune tone.

Future research should investigate signaling and effector networks, including afferent pathways and circuits by which GI signals lead to the diagnostic symptoms of DGBI in motility, 5-HT, and pain, as well as common comorbidities, such as stress and mood dysfunction, to ask questions such as "Are there common signals in different subgroups of IBS?" Using fecal microbial transplants to generate new experimental models recapitulating symptom profiles that are seen in subsets of patients with DGBI will likely yield exciting new observations to help delineate and target, for example, microbial mechanisms underpinning psychiatric comorbidity in DGBI. Such data will enable scientists and clinicians to answer questions like "How can we modulate the gut microbiota, or the immune or nervous systems to treat DGBI more effectively?"

## References


1. Gazouli M, Wouters MM, Kapur-Pojskic L, et al. Lessons learned—resolving the enigma of genetic factors in IBS. Nat Rev Gastroenterol Hepatol 2016;13:77–87.
2. Morarach K, Mikhailova A, Knoflach V, et al. Diversification of molecularly defined myenteric neuron classes revealed by single-cell RNA sequencing. Nat Neurosci 2021;24:34–46.
3. Nikolaev M, Mitrofanova O, Broguiere N, et al. Homeostatic mini-intestines through scaffold-guided organoid morphogenesis. Nature 2020;585:574–578.
4. Marklund U. Diversity, development and immunoregulation of enteric neurons. Nat Rev Gastroenterol Hepatol 2022;19:85–86.
5. Fawkner-Corbett D, Antanaviciute A, Parikh K, et al. Spatiotemporal analysis of human intestinal development at single-cell resolution. Cell 2021;184:810–826.e23.
6. Sonnenburg JL. Microbiome engineering. Nature 2015; 518:S10.
7. Ramadi KB, Srinivasan SS, Traverso G. Electroceuticals in the gastrointestinal tract. Trends Pharmacol Sci 2020; 41:960–976.
8. Yang B, Treweek JB, Kulkarni RP, et al. Single-cell phenotyping within transparent intact tissue through whole-body clearing. Cell 2014;158:945–958.
9. Grundy L, Erickson A, Brierley SM. Visceral pain. Annu Rev Physiol 2019;81:261–284.

10. Bayrer JR, Castro J, Venkataraman A, et al. Gut enterochromaffin cells drive visceral pain and anxiety. Nature 2023;616:137–142.
11. Greenwood-Van Meerveld B, Johnson AC. Mechanisms of stress-induced visceral pain. J Neurogastroenterol Motil 2018;24:7–18.
12. Harrington AM, Castro J, Erickson A, et al. Chapter 17—Extrinsic sensory afferent nerves innervating the gastrointestinal tract in health and disease. In: Said HM, ed. Physiology of the Gastrointestinal Tract. (Sixth Edition). Cambridge: Academic Press; 2018:387–418.
13. Grove JCR, Knight ZA. The neurobiology of thirst and salt appetite. Neuron 2024;112:3999–4016.
14. Hockley JRF, Taylor TS, Callejo G, et al. Single-cell RNAseq reveals seven classes of colonic sensory neuron. Gut 2019;68:633–644.
15. Wolfson RL, Abdelaziz A, Rankin G, et al. DRG afferents that mediate physiologic and pathologic mechanosensation from the distal colon. Cell 2023;186:3368–3385.e18.
16. Servin-Vences MR, Lam RM, Koolen A, et al. PIEZO2 in somatosensory neurons controls gastrointestinal transit. Cell 2023;186:3386–3399.e15.
17. Morales-Soto W, Gonzales J, Jackson WF, et al. Enteric glia promote visceral hypersensitivity during inflammation through intercellular signaling with gut nociceptors. Sci Signal 2023;16:eadg1668.
18. Orock A, Louwies T, Ligon CO, et al. Environmental enrichment prevents stress-induced epigenetic changes in the expression of glucocorticoid receptor and corticotrophin releasing hormone in the central nucleus of the amygdala to inhibit visceral hypersensitivity. Exp Neurol 2021;345:113841.
19. Sharkey KA, Mawe GM. The enteric nervous system. Physiol Rev 2023;103:1487–1564.
20. Seguella L, Gulbransen BD. Enteric glial biology, intercellular signalling and roles in gastrointestinal disease. Nat Rev Gastroenterol Hepatol 2021;18:571–587.
21. Sanders KM, Drumm BT, Cobine CA, et al. Ca(2+) dynamics in interstitial cells: foundational mechanisms for the motor patterns in the gastrointestinal tract. Physiol Rev 2024;104:329–398.
22. Brierley SM, Linden DR. Neuroplasticity and dysfunction after gastrointestinal inflammation. Nat Rev Gastroenterol Hepatol 2014;11:611–627.
23. Schemann M, Frieling T, Enck P. To learn, to remember, to forget-How smart is the gut? Acta Physiol (Oxf) 2020;228:e13296.
24. Aguilera-Lizarraga J, Florens MV, Viola MF, et al. Local immune response to food antigens drives meal-induced abdominal pain. Nature 2021;590:151–156.
25. Godinho-Silva C, Cardoso F, Veiga-Fernandes H. Neuro-immune cell units: a new paradigm in physiology. Annu Rev Immunol 2019;37:19–46.
26. Rosas-Ballina M, Olofsson PS, Ochani M, et al. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. Science 2011;334:98–101.
27. Spencer NJ, Hu H. Enteric nervous system: sensory transduction, neural circuits and gastrointestinal motility. Nat Rev Gastroenterol Hepatol 2020;17:338–351.
28. Muller PA, Koscso B, Rajani GM, et al. Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. Cell 2014;158:300–313.
29. Matheis F, Muller PA, Graves CL, et al. Adrenergic signaling in muscularis macrophages limits infection-induced neuronal loss. Cell 2020;180:64–78.e16.
30. Klose CS, Artis D. Innate lymphoid cells as regulators of immunity, inflammation and tissue homeostasis. Nat Immunol 2016;17:765–774.
31. Yu LW, Agirman G, Hsiao EY. The gut microbiome as a regulator of the neuroimmune landscape. Annu Rev Immunol 2022;40:143–167.
32. O'Riordan KJ, Moloney GM, Keane L, et al. The gut microbiota-immune-brain axis: therapeutic implications. Cell Rep Med 2025;6:101982.
33. Nikolova VL, Hall MRB, Hall LJ, et al. Perturbations in gut microbiota composition in psychiatric disorders: a review and meta-analysis. JAMA Psychiatry 2021;78:1343–1354.
34. Gheorghe CE, Ritz NL, Martin JA, et al. Investigating causality with fecal microbiota transplantation in rodents: applications, recommendations and pitfalls. Gut Microbes 2021;13:1941711.
35. Keane L, Clarke G, Cryan JF. A role for microglia in mediating the microbiota-gut-brain axis. Nat Rev Immunol 2025;25:847–861.
36. Haas-Neill S, Forsythe P. A budding relationship: bacterial extracellular vesicles in the microbiota-gut-brain axis. Int J Mol Sci 2020;21:8899.
37. Greenwood-Van Meerveld B, Johnson AC. Stress-induced chronic visceral pain of gastrointestinal origin. Front Syst Neurosci 2017;11:86.
38. Margolis KG, Cryan JF, Mayer EA. The microbiota-gut-brain axis: from motility to mood. Gastroenterology 2021;160:1486–1501.
39. Brierley SM, Greenwood-Van Meerveld B, Sarnelli G, et al. Targeting the endocannabinoid system for the treatment of abdominal pain in irritable bowel syndrome. Nat Rev Gastroenterol Hepatol 2023;20:5–25.
40. Vanuytsel T, Bercik P, Boeckxstaens G. Understanding neuroimmune interactions in disorders of gut-brain interaction: from functional to immune-mediated disorders. Gut 2023;72:787–798.
41. Vanner S, Greenwood-Van Meerveld B, Mawe G, et al. Fundamentals of neurogastroenterology: basic science. Gastroenterology 2016;150:1280–1291.
42. Margolis KG, Li Z, Stevanovic K, et al. Serotonin transporter variant drives preventable gastrointestinal abnormalities in development and function. J Clin Invest 2016;126:2221–2235.
43. Staudacher HM, Mikocka-Walus A, Ford AC. Common mental disorders in irritable bowel syndrome: pathophysiology, management, and considerations for future randomised controlled trials. Lancet Gastroenterol Hepatol 2021;6:401–410.
44. Zia JK, Lenhart A, Yang PL, et al. Risk Factors for abdominal pain-related disorders of gut-brain interaction in adults and children: a systematic review. Gastroenterology 2022;163:995–1023.e3.
45. Foong JPP, Hung LY, Poon S, et al. Early life interaction between the microbiota and the enteric nervous system.

Am J Physiol Gastrointest Liver Physiol 2020; 319:G541–G548.
46. Najjar SA, Hung LY, Margolis KG. Serotonergic control of gastrointestinal development, motility, and inflammation. Compr Physiol 2023;13:4851–4868.
47. Binienda A, Storr M, Fichna J, et al. Efficacy and safety of serotonin receptor ligands in the treatment of irritable bowel syndrome: a review. Curr Drug Targets 2018;19:1774–1781.

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**Conflicts of interest**
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