Irritable Bowel Syndrome: Proposed Mechanisms of Pathophysiology and the Underlying Dysregulation of Brain-Gut Interaction

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Abstract

Irritable bowel syndrome (IBS) is a disease of the gastrointestinal tract affecting approximately 11-21% of people worldwide (Xiao et al., 2021). This research focuses on the dysregulation of gut homeostasis in IBS and explains how the pathophysiology of the gut is controlled by the bidirectional brain-gut axis. The mechanisms of the brain-gut axis consist of neuro-immune cross talk, neuro-hormone cross talk, microbiome-gut signaling, and the HPA-axis. Each of these contribute to one or more of the possible phenotypes of gut pathophysiology, which could include intestinal permeability, visceral hypersensitivity, and motility disturbance (Xiao et al., 2021). Stress is also an important peripheral contributor to IBS through many of these mechanisms, especially the hypothalamic-pituitary-adrenal (HPA)-axis (Camilleri et al., 2012). The goal of this research is to present a more comprehensive pathophysiology of IBS to give a better foundation for future research and treatment. This thesis is limited to and relies on previously published primary research but will link together underlying causes of IBS within the brain-gut axis to propose manners of future targeted treatments.

Irritable Bowel Syndrome: Proposed Mechanisms of Pathophysiology and the Underlying Dysregulation of Brain-Gut Interaction

The irritability of irritable bowel syndrome (IBS) is well known to the general population by its debilitating symptoms and overall prominence in humans across the globe. It is a functional gastrointestinal disorder characterized by chronic abdominal pain, bloating, and altered bowel habits, including diarrhea and constipation (Saha, 2014). The etiology of IBS is multifactorial and includes genetic, environmental, and psychological factors (Saha, 2014). There is a wide range of symptoms involved in this disorder that studies have shown to be highly heterogenous (Van Thiel et al., 2020). The most common symptoms of IBS include abdominal pain or discomfort, bloating, and altered bowel habits, including diarrhea, constipation, or both (Saha, 2014). The pain or discomfort is usually located in the lower abdomen and is often relieved by defecation (Saha, 2014). In addition to these primary symptoms, patients with IBS can experience a range of secondary symptoms, including nausea, fatigue, and headaches (Saha, 2014). Some patients may also experience extra-intestinal symptoms, such as urinary symptoms, sexual dysfunction, and musculoskeletal pain (Saha, 2014). The diagnosis of IBS is made based on the presence of symptoms and the exclusion of organic gastrointestinal diseases (Saha, 2014). One manner of separating these symptoms and pathologies for diagnosis is by subclassifying each patient according to their dysfunctional bowel habits, as diarrhea (IBS-D), constipation (IBS-C), or mixed type (IBS-M), all of which are associated with gut pain (Saha, 2014). Post infectious IBS (IBS-PI) has also been identified to describe an onset of IBS following infection that is highly correlated with inflammation related symptoms (Camilleri et al., 2012). IBS-D has been shown to be attributed, in part, by the acceleration of colonic transit, which does not allow for the needed reabsorption of water in the colon (Camilleri et al., 2014). The opposite is seen in

IBS-C, where the deceleration of colonic transit causes an overabsorption of water in the colon (Camilleri et al., 2014). The Rome IV criteria, which are widely used in clinical practice, define IBS as recurrent abdominal pain or discomfort at least one day per week in the last three months, associated with two or more of the following: improvement with defecation, onset associated with a change in frequency of stool, and onset associated with a change in form (appearance) of stool (Saha, 2014). There is a wide variety of possible indications of IBS. Therefore, a multitude of possible mechanisms could contribute to IBS pathophysiology. The large number of factors that play a role in the pathophysiology of IBS make exact causative mechanisms difficult to identify, since the influence of multiple cellular mechanisms can be different for each patient. This also makes reproducibility of studies a challenge. It has been found that many factors take part in the variety of manifestations of IBS, some of which include neural sensitivity, motility disturbance due to signaling, a change in intestinal permeability, and alterations of the gut microbiota (Videlock et al., 2018).

Visceral hypersensitivity and motility disturbance have been highly studied because they are more easily observed pathologies of IBS. In the case of IBS, motility disturbance can occur as either elevated or reduced contractions of the gut, giving more complexity to the current and possible experimentations as well as causes of the disease (Houghton et al., 2007). Intestinal permeability is key to studying the ability of the GI tract to properly absorb nutrients, which is increased or decreased, once again, depending on the subclass of IBS, and adding to the complexity of causes (Camilleri et al., 2014). The importance of the gut microbiota in the maintenance and development of a healthy gut has been more recently discovered (O'Malley, 2016). The difference and lack of biodiversity in IBS patients has been identified and studied as another important factor of abnormal function of the gut.

The main underlying mechanisms encompassing these factors are a part of the brain-gut axis, which involve bidirectional signaling between both the brain and gut that is necessary for normal gut function (O'Malley, 2016). Abnormal function of this pathway has been shown to contribute to each of the abnormal factors associated with IBS including, visceral neural sensitivity, colonic motor function, secretory function, and permeability (O'Malley, 2016). The brain-gut axis consists of neuro-immune crosstalk, neuro-hormone cross talk, microbiome-gut signaling, and the HPA-axis (O'Malley, 2016). Each of these mechanisms contribute in part to the factors found in IBS. Neuro-immune crosstalk in IBS involves low-grade inflammation of the gut and is thought to result in dysregulation of gut permeability as well as visceral hypersensitivity found in IBS (O'Malley, 2016). Neuro-hormone crosstalk is thought to be localized hormonal interaction within the gut and involved in the motility disturbance of the gut in IBS (O'Malley, 2016). Microbiome-gut signaling in IBS is implicated in alterations of permeability, visceral hypersensitivity, and motility, but it's role has only recently begun to be studied(Bhattarai et al., 2017). The last portion of the brain-gut signaling axis is the HPA axis, which is the primary neuroendocrine system that maintains homeostasis in response to stress and contributes to each of the factors mentioned (Myers & Greenwood-Van Meerveld, 2010; O'Malley, 2016). This is important because of the high comorbidity noted between stress-associated mood disorders and IBS, meaning that heightened stress can be conducive to worsened symptoms of IBS (O'Malley, 2016). The regulation of the gut in this way gives insight into the dysregulation of the nervous system and its communication with the gastrointestinal tract.

The study and collection of data on causative factors of IBS, and current studies examining possible treatments, allow for the future identification of foundational mechanisms

that could be altered to discover new, groundbreaking treatments. Treatment options and therapies are discussed following detailed descriptions of each factor of IBS in order to outline the purpose of individual research, topics for future research, and possibilities of relief for patients with IBS. The aim of this research is the compilation of existing research on pathological mechanisms caused by underlying peripheral and internal factors of the GI tract to gain an understanding of the pathophysiology of the brain-gut interaction and possible targeted treatment options for IBS patients.

Visceral Hypersensitivity

The Normal Brain-gut Axis and Perception of Pain

The gastrointestinal (GI) tract has a complex network of afferent and efferent neurons that regulate the sensory and motor functions of the gut (Videlock et al., 2018). These neurons are located in the enteric nervous system (ENS) and the spinal cord, and they transmit information from the gut to the central nervous system (CNS) (Videlock et al., 2018). The ENS is a complex network of neurons and glial cells that regulate the functions of the gastrointestinal tract (Videlock et al., 2018). It is also known as the second brain because it can function independently of the CNS (Videlock et al., 2018). The ENS is principally composed of the myenteric plexus and submucosal plexus (Nezami & Srinivasan, 2010). The myenteric plexus is located between the circular and longitudinal layers of the smooth muscle in the GI tract and is responsible for relaxation and contraction of the intestinal wall (Nezami & Srinivasan, 2010). The submucosal plexus is responsible for sensing the environment of the lumen to then regulate blood flow of the intestines, epithelial cell function and gastrointestinal secretion (Nezami & Srinivasan, 2010).

The ENS receives sensory input from the gut through several types of sensory neurons (Videlock et al., 2018). These neurons have specialized receptors that detect changes in the gut environment and transmit this information to the CNS (Videlock et al., 2018). The primary afferent neurons in the ENS are categorized as mechanoreceptors or chemoreceptors (Myers & Greenwood-Van Meerveld, 2010; Videlock et al., 2018). Mechanoreceptors respond to mechanical stimuli, such as distension, while chemoreceptors respond to chemical stimuli, such as nutrients and toxins (Myers & Greenwood-Van Meerveld, 2010; Videlock et al., 2018).

Once the sensory information is transmitted to the CNS, it is processed and integrated in several regions of the brain, including the amygdala, prefrontal cortex, and insula (Myers & Greenwood-Van Meerveld, 2010; Videlock et al., 2018). These brain regions are involved in the processing of pain, emotion, and visceral perception (Myers & Greenwood-Van Meerveld, 2010; Videlock et al., 2018). The integration of sensory information from the gut and other parts of the body results in the perception of abdominal pain and discomfort (Myers & Greenwood-Van Meerveld, 2010; Videlock et al., 2018).

Introduction to Visceral Hypersensitivity

One of the main characteristics of IBS is hypersensitivity of the lower GI tract. Visceral hypersensitivity is known as the factor causing increased pain sensation in most IBS patients (Van Thiel et al., 2020). It refers to increased sensitivity to normal gut stimuli, such as distension, which can lead to abdominal pain and discomfort (Van Thiel et al., 2020). Studies have shown that patients with IBS have increased sensitivity to gut stimuli compared to healthy individuals (Van Thiel et al., 2020). Visceral hypersensitivity is thought to be due to alterations in the sensory pathways of the brain-gut signaling axis, which sends and receives neuronal signals to and from the gut and the brain (Van Thiel et al., 2020). The components of this

signaling axis have been shown to be causes of visceral hypersensitivity could include dysregulated neuro-immune crosstalk, the HPA-axis, and microbiome-gut signaling.

The exact mechanisms underlying visceral hypersensitivity are not fully understood but may involve alterations in the expression of ion channels and receptors in sensory neurons (Van Thiel et al., 2020). Peripheral nociceptors, along with a diverse range of signaling pathways and ion channels innervating the bowel, are thought to be hypersensitized by luminal contents of the bowel in IBS as well as distention (Van Thiel et al., 2020). The trigger for visceral hypersensitivity in IBS is thought to be stress, which could be causative of each of the mechanisms that play a role in the development of IBS (Van Thiel et al., 2020). Corticotropin-releasing factor (CRF), a mediator released in response to stress, plays a crucial role initiating visceral hypersensitivity through the HPA-axis and neuro-immune crosstalk (Van Thiel et al., 2020).

Visceral Hypersensitivity via Abnormal Neuro-immune Crosstalk

CRF receptors are expressed on immune cells throughout the Gastrointestinal tract, indicating the connection of stress-signaling molecules and intestinal immune responses (Van Thiel et al., 2020). Due to this expression of receptors on immune cells, one way of developing visceral hypersensitivity in IBS has been found to be due to stress-induced mast cell signaling (Van Thiel et al., 2020). Mucosal mast cells in the colon can be activated by CRF, which triggers the release of proinflammatory mediators including histamine, proteases, and cytokines (O'Malley, 2016; Van Thiel et al., 2020). This process of mast cell degranulation has been identified as mediating visceral nociception in IBS patients, initiating low-grade inflammation in the mucosa of the gut, and increasing the excitability of afferent endings innervating the colon

(O'Malley, 2016; Van Thiel et al., 2020). The mediators released from mast cells play a crucial role in visceral hypersensitivity, especially neuronal histamine signaling (Van Thiel et al., 2020).

Neuronal histamine signaling is indicated in the sensitization of transient receptor potential (TRP) channels located in the rectal submucosal plexus of IBS patients (Balemans et al., 2019). TRP channel function can also be induced by proinflammatory factors other than histamine, but neuronal histamine signaling is thought to be a major contributor to heightened visceral pain perception associated with IBS (Balemans et al., 2019). TRP channels are a group of ion channels that are activated by various stimuli, including temperature, pressure, and chemical stimuli (Balemans et al., 2019). They are expressed in sensory neurons and play a role in the detection and transmission of sensory information to the ENS (Balemans et al., 2019). Several types of TRP channels have been implicated in the development of visceral hypersensitivity, including TRPV1, TRPV4, TRPA1, and TRPM8 (Balemans et al., 2019). Histamine's effects on sensitization of TRP channels located in the submucosa of the gastrointestinal tract is evidenced to be done via activation of histamine 1 receptor (H_1R) (Balemans et al., 2019; Van Thiel et al., 2020).

Visceral Hypersensitivity via the HPA-axis

Also activated by the synthesis and release of CRF in response to perceived stressors, is the HPA-axis (O'Malley, 2016). CRF in the CNS, as opposed to peripheral expression, is also indicated in IBS and crucial to pain modulation in the GI tract (Myers & Greenwood-Van Meerveld, 2010). Specific to CRF effects on visceral nociception is its release into the central nucleus of the amygdala (CeA) (Myers & Greenwood-Van Meerveld, 2010). Patients with IBS have shown enhanced activation of the amygdala compared to healthy controls in response to visceral stimulation through neuroimaging studies (Yuan et al., 2021). The significance of CRF

in the CNS is the stimulation of the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary to then stimulate the release of corticosteroids, including cortisol, from the adrenal gland (Myers & Greenwood-Van Meerveld, 2010). Cortisol has been found to be elevated in IBS patients both under stress and at baseline compared to controls (Myers & Greenwood-Van Meerveld, 2010). Circulating cortisol binds to glucocorticoid receptors (GR) in multiple brain regions to stop the stress response (Yuan et al., 2021). When binding to GRs in the amygdala, however, elevated cortisol facilitates the stress response rather than stopping it, thus causing visceral sensitivity (Yuan et al., 2021). One possible signaling pathway implicated in this facilitating response that has recently been studied is synaptic engulfment in the CeA mediated by microglia cells (Yuan et al., 2021). This microglial remodeling of the amygdala leads to visceral hypersensitivity (Yuan et al., 2021). A recent study has provided evidence for this pathway in which C1q/C3-CR3 signaling is used for synaptic engulfment in response to chronic stress (Yuan et al., 2021). C1q/C3-CR3 signaling is the classical complement cascade and triggers microglial mediated phagocytosis (Yuan et al., 2021). This cascade has been associated with the immune response following stress and contributing to amygdala neural plasticity (Yuan et al., 2021). Elevated expression levels of complement within this classical cascade cause increased engulfment by the microglia, which then causes visceral hypersensitivity (Yuan et al., 2021).

Altered Intestinal Permeability

Normal Intestinal Permeability

Intestinal permeability refers to the ability of the intestinal mucosa to control the passage of substances between the lumen of the gut and the bloodstream (Camilleri et al., 2012). The healthy intestinal barrier is a series of defensive layers providing a “gated wall” that stands

between the luminal contents of the gut and everything else in the body (Camilleri et al., 2012). The lumen is the first line of defense that breaks down bacteria and antigens using gastric acids and pancreatic and biliary secretions (Camilleri et al., 2012). The lumen is also home to bacteria that work to inhibit colonization of pathogens (Camilleri et al., 2012). Along the walls of the lumen is the microclimate that consists mostly of the mucus layer and prevents pathogens from adhering to the epithelium (Camilleri et al., 2012). The next layer is the epithelium, which acts as a physical barrier of cells joined together by tight junctions and reacts to stimuli by releasing antimicrobial peptides and chloride secretions (Camilleri et al., 2012). Surrounding the epithelium is the lamina propria, which houses the ENS, immune cells, endocrine system, and other components (Camilleri et al., 2012). Altogether, this barrier is made to allow diffusion of only small lipid-soluble particles and some lipophobic molecules while using transcellular and paracellular transport for other substances (Camilleri et al., 2012). Small hydrophilic molecules are transported paracellularly via tight junctions that are highly regulated and able to form a tight seal in healthy mucosa (Camilleri et al., 2012). Tight junctions (TJ) form the paracellular gateway, while ion channels are a large contributor to the transcellular gateway (Camilleri et al., 2012). Important to note for this paper is the role the protein occludin plays in the maintenance of these TJs (Camilleri et al., 2012). Also integral to the maintenance of the intestinal barrier is ion channels (Camilleri et al., 2012). This is evident by chloride channel protein 2 (CIC2), which may promote the presence of occludin in the membrane, therefore playing a critical part in modulating the tight junction barrier in the intestines (Camilleri et al., 2012).

Altered Intestinal Permeability in IBS

An alteration in intestinal permeability, commonly known as "leaky gut," has been proposed as a potential mechanism contributing to the pathogenesis of irritable bowel syndrome

(IBS) (Camilleri et al., 2012). Intestinal permeability is altered in IBS patients and shown to be caused by a dysregulation of ion channels and other structures in the intestinal epithelial cell monolayers (Camilleri et al., 2012).

A recent meta-analysis found that patients with IBS have significantly increased levels of serum zonulin, a protein that regulates tight junctions in the gut, compared to healthy controls (Camilleri et al., 2012). Zonulin levels were significantly higher in IBS patients with diarrhea-predominant symptoms compared to those with constipation-predominant symptoms (Camilleri et al., 2012).

Neuro-immune Crosstalk Effect on Permeability

Just as stress triggers visceral hypersensitivity, stress also is thought to trigger altered permeability in IBS (Camilleri et al., 2012). This appears to be done by CRF once again, through the stimulation of mast cells to release nerve growth factor (NGF), cytokines, and other proinflammatory molecules (Camilleri et al., 2012). CRF is implicated in the alteration of transcellular and paracellular permeability by this mechanism (Camilleri et al., 2012). It is believed that alterations in gut permeability can lead to increased exposure to luminal antigens, triggering an immune response and low-grade inflammation in the gut (Camilleri et al., 2012). In both IBS-C and IBS-D patients, a disruption of expression and distribution of tight junction protein structures were noted, decreasing the cell-cell adhesion of the epithelial monolayer significantly (Camilleri et al., 2012). The presence of increased intestinal permeability alters epithelial barrier function, allowing for pathogens and other harmful substances within the intestines to cause the known symptoms of IBS (Camilleri et al., 2012).

Genetic Alterations Affecting Permeability

Multiple mitochondrial genes have been shown to be under or over-expressed in IBS contributing to its dysregulation (Camilleri et al., 2012). IBS-D specifically, has shown an under expression of genes affecting the normal epithelial layer structure in the intestines, therefore weakening the mucosal barrier function, and increasing mucosal permeability (Camilleri et al., 2012). An overexpression of genes leading to increased fluid secretion in the intestines of IBS-D patients has also been studied, possibly involving two mechanisms of excretion (Camilleri et al., 2012). One mechanism involves goblet cells secreting an overabundance of endogenous ligands, which bind to guanylate cyclase C receptors on the ion channels of enterocytes causing increased secretion of chloride ions into the intestine (Camilleri et al., 2012). This increased secretion involves the alteration of the intestinal barrier, meaning the permeability of the barrier is increased (Camilleri et al., 2012).

Motility Disturbance

IBS is also characterized by a previously mentioned abnormality of smooth muscle colonic motility (Houghton et al., 2007). Abnormalities in gut motility have been observed in patients with IBS, including increased colonic transit time, impaired motor function, and altered reflex responses (Houghton et al., 2007).

Normal Gut Motility

GI tract motility is a key aspect of its function and the mechanosensitive cells that regulate this process are present in all layers throughout the wall of the GI tract (Alcaino et al., 2017). This includes the mucosa, sub mucosa, smooth muscle, and submucosal and myenteric plexuses (Alcaino et al., 2017).

Normal Serotonin Signaling

On the surface of the mucosa, enterochromaffin (EC) cells as well as some enteric neurons detect mechanical and chemical forces and synthesize and release serotonin (5-HT), which is important for normal secretion, sensation, and motility within the GI tract (Alcaino et al., 2017). The serotonin released from EC cells or enteric neurons is known to have many functions within the GI tract due to its modulation of the ENS as well as possible modulation of the CNS via signals activating sensory afferent connections (Del Colle et al., 2020). Within the mucosa, submucosa, and muscle layers, are the intrinsic and extrinsic neurons of the ENS that are responsible for regulating GI mechanosensation (Alcaino et al., 2017). Enteric neurons respond to mechanical stress in the tract wall, not only playing a large role in visceral sensitivity, but also using a control reflex to propagate peristalsis (Alcaino et al., 2017). Therefore, modulation of the ENS and CNS by serotonin encompasses the effect serotonin signaling has on peristalsis reflexes and the sensation of pain or discomfort (Del Colle et al., 2020).

Production of serotonin within the EC cells and enteric neurons is regulated by isoforms of the rate-limiting enzyme, tryptophan hydroxylase (TPH) (Del Colle et al., 2020). Prior to release, serotonin is formed and packaged into secretory vesicles (Del Colle et al., 2020). Following a stimulus via mechanical stimulation to the EC cell or presynaptic enteric neuron, the vesicular monoamine transporter 1 (VMAT1) carries the secretory vesicles to the apical and basolateral membranes for fusion with the plasma membrane. Serotonin is then released into the mucosal lumen (Chin et al., 2012; Del Colle et al., 2020). Once it is secreted into the lumen of the GI tract, the mucosal serotonin reaches 5-HT receptors on the mucosal projecting neurons and epithelium to initiate its effects (Alcaino et al., 2017). The concentration of 5-HT in the lumen is regulated primarily by the serotonin reuptake transporter (SERT), meaning this

transporter regulates the availability of 5-HT for neurotransmission and enhancing reflex activity (Del Colle et al., 2020). SERT transports the serotonin back into the presynaptic neuron or EC cell, where it is inactivated by monoamine oxidase or repackaged into vesicles for future release (Del Colle et al., 2020).

5-HT's effects on motility are known to be primarily through the activation of two receptors named 5-HT_3 and 5-HT_4 (Del Colle et al., 2020). These receptors are found on the myenteric and submucosal plexuses of the ENS, intrinsic and extrinsic sensory neurons, and EC cells within the GI tract (Del Colle et al., 2020). Activation of these receptors result in the release of acetylcholine at the synaptic cleft of excitatory enteric neurons and consequently, smooth muscle contraction (Nezami & Srinivasan, 2010). 5-HT is also able to activate inhibitory enteric or nitrergic neurons that release NO, causing relaxation of smooth muscle through activation of 5-HT_1 or some 5-HT_4 receptors (Nezami & Srinivasan, 2010). This means that depending on the target of 5-HT, excitation or relaxation of the peristaltic reflex may occur in a specific area of the GI tract (Nezami & Srinivasan, 2010).

Other Mechanisms of Motility

Contained in the smooth muscle layer of the GI tract are the smooth muscle cells (SMCs), as well as the interstitial cells of cajal (ICCs), which together regulate the contractions in response to "slow waves" apart from the enteric nervous system (Alcaino et al., 2017). SMCs function to convert electrical energy into contractions within the GI tract by using calcium and sodium voltage-gated ion channels (Faville et al., 2008). The "pacemakers" of SMCs are ICCs, which connect to the SMCs by gap junctions and initiate the release of calcium ions into the SMCs to cause contraction of the muscle (Faville et al., 2008). The formation of slow waves is accomplished by unitary potentials (UPs) that are the small and localized SMC membrane

fluctuations able to move down the GI muscles (Faville et al., 2008). The movements of the large intestine are haustral contractions that move chyme slowly through the segments of haustra present, and mass movement, which pushes chyme quickly towards the rectum (Volk & Lacy, 2017). The descending colon stores the waste until emptied into the rectum by the sigmoid colon (Volk & Lacy, 2017). From the rectum, the waste is stored until the it can be eliminated from the body through the anus, by the process of defecation (Volk & Lacy, 2017). All the differing types of motilities in the GI tract work in unison to mechanically digest nutrients and excrete waste.

Abnormal Serotonin Signaling

Motility disturbance in IBS is possibly influenced by abnormal serotonin (5-HT) concentrations within the blood (Houghton et al., 2007). The abnormal secretion of 5-HT is contributed to an abnormal expression of the T-type calcium channels involved in the cAMP-PKA signaling pathway (Videlock et al., 2018). IBS-D patients have been characterized as having highly elevated levels of plasma 5-HT compared to healthy individuals in both the fasted and fed states, whereas IBS-C patients have shown reduced concentrations in the fed condition compared to healthy individuals (Houghton et al., 2007). The release of elevated concentrations of 5-HT from the enterochromaffin cells of the intestines increase the frequency of both propagating contractions, and high amplitude propagating contractions (Houghton et al., 2007). The opposite is shown by limited or lack of 5-HT, where motility of the gut is reduced (Houghton et al., 2007). The consequences of these motility disturbances, therefore, are sped digestive function or slowed digestion, sequentially.

Influence of the Brain-Gut Axis

Abnormalities in gut motility may be due to alterations in the ENS, possibly caused by altered concentrations of 5-HT. Studies have shown that patients with IBS have alterations in the

ENS, including increased numbers of enteric neurons, abnormal neurotransmitter signaling, and altered expression of neuropeptides (Volk & Lacy, 2017).

Microbiota Dysbiosis

Gut microbiota refers to the diverse community of microorganisms that inhabit the human gut and have also been implicated in the pathogenesis of IBS (Bhattarai et al., 2017).

Normal Gut Microbiota

An important aspect of the gut's function involves the abundance and diversity of intestinal microbiota. Although the duodenum and jejunum of the small intestine do not harbor large numbers of bacteria, the ileum of the small intestine, as well as the large intestine, are colonized by an abundance and variety of microbes (Vuik et al., 2019). The intestinal bacteria aid in a wide variety of systems within the intestine to maintain its homeostasis (Parker et al., 2018). The mucosal immune system is matured and made adaptive by the presence of bacterial antigens (Parker et al., 2018). Microbiota of the gut also play a large role in the functional and morphological maturation of the enteric nervous system, due to electrical signaling, and release of neurotransmitters, or neurotoxins from the bacteria (Parker et al., 2018). Overall, the microbiota heavily influences the homeostasis of the intestines through crosstalk known as microbiome-gut signaling, allowing the involved systems to mature and develop (Parker et al., 2018).

Microbiota Effects on Brain-gut Axis

Recent evidence has been given for the modulation of the brain-gut axis by intestinal microbiota and their products (Bhattarai et al., 2017). One study found that postnatal gut microbial colonization can regulate stress response by programing the HPA-axis during development (Bhattarai et al., 2017). There has also been research focused on gut bacteria's

mechanisms of alteration of brain function and behavior (Bhattarai et al., 2017). One mechanism that is known is through free fatty acid production (Bhattarai et al., 2017). A likely example of this is propionic acid, which is a product of gut bacteria (Bhattarai et al., 2017). Propionic acid can easily cross the blood-brain barrier, giving it the ability to access the brain to influence its behavior and function (Bhattarai et al., 2017). Some gut bacteria, such as *Lactobacilli* and *Bifidobacteria*, can also produce gamma-amino butyric acid (GABA), which is an inhibitory neurotransmitter within the human brain; therefore the production of this as a gut microbiota metabolite could be linked to modulation of the brain-gut axis (Bhattarai et al., 2017).

Microbiota Effects on Visceral Sensation

In healthy individuals, gut microbiota may be involved in normal visceral sensation (Bhattarai et al., 2017). It has been noted that some bacteria, including *Lacto bacillus reuteri* could be involved (Bhattarai et al., 2017). *Lacto bacillus reuteri* has been shown to signal TRPV1 exhibiting an antinociceptive effect in the gut (Bhattarai et al., 2017). This could indicate the gut bacteria's potential role in maintaining normal visceral sensation as well as visceral hypersensitivity in IBS (Bhattarai et al., 2017).

Microbiota Effects on Intestinal Barrier Permeability

It is well known that gut microbiota exhibit important actions in maintaining the epithelial barrier of the GI tract (Bhattarai et al., 2017). Short-chain fatty acids (SCFAs), derived from bacteria, help to maintain the structure and function of the epithelial barrier (Bhattarai et al., 2017). One SCFA that has been studied is butyrate, which has been shown to increase the expression of TJ proteins, including occludins, claudins, and zonula occludens proteins (Bhattarai et al., 2017). It has been suggested that early exposure to bacteria could promote the development and maturation of the GI tract epithelial layer (Bhattarai et al., 2017).

Another aspect of gut bacteria's effects on permeability is seen in its protection of the mucus layer of the GI tract (Bhattarai et al., 2017). Gut bacteria within the mucosa of the GI tract aid in protection from pathogens, keeping them from accessing the epithelial surface (Bhattarai et al., 2017). Composition and thickness of the mucus layer is highly indicative of possible inflammatory responses (Bhattarai et al., 2017). Meaning, detection of pathogens within this layer, or a change in composition of the layer, could lead to inflammation (Bhattarai et al., 2017). Therefore, probiotics' protection can also be seen in the upregulation of mucus secretion within the GI tract (Bhattarai et al., 2017).

Microbiota Effects on 5-HT Signaling

In recent studies, it has been shown that microbiota are able to communicate with the host, specifically via the serotonergic system, using cells that are thought to regulate immunity and GI motility in the GI tract (Kwon & Khan, 2022). These cells are enteroendocrine cells (EECs), of which EC cells are a subset (Kwon & Khan, 2022). 5-HT is therefore now thought to be an important connection between the microbiome and its host (Kwon & Khan, 2022). Gut microbes are essential to the synthesis of 5-HT, primarily through microbial metabolites that signal the EC cells to generate 5-HT (Kwon & Khan, 2022). Not only are the microbes responsible for host serotonin signaling, but the host 5-HT is also responsible for microbial composition, making the host-microbiome signaling bidirectional (Kwon & Khan, 2022). One way that this is shown is using quorum sensing (QS) to indirectly affect the abundance of some microbial species in the gut (Kwon & Khan, 2022). QS is cell to cell signaling between bacteria, initiated in response to changes in the local environment, and controlling bacterial processes such as biofilm formation and virulence factor expression (Kwon & Khan, 2022). It was discovered that 5-HT increases biofilm formation and virulence of *Pseudomonas aeruginosa* in the gut

through activation of this system (Kwon & Khan, 2022). Another example recently provided is that mucosal 5-HT can dephosphorylate the histidine sensor kinase CpxA to decrease the expression of virulence genes in human enteric pathogen enterohemorrhagic *E. coli* (EHEC) and the murine pathogen *Citrobacter rodentium* (Kwon & Khan, 2022). These findings support the assertion that 5-HT signaling, and the gut microbiota interactions are important in maintaining homeostasis of the GI tract (Kwon & Khan, 2022).

Lack of Microbiota Diversity in IBS

There has been a lack of microbiota diversity found within the gut of patients with IBS (Bhattarai et al., 2017). Studies have shown that patients with IBS have a distinct gut microbiota composition compared to healthy individuals (Carroll et al., 2011). The alteration of this biodiversity can significantly influence the functions of the brain-gut axis, intestinal barrier function, immune activation, and gastrointestinal motility (Bhattarai et al., 2017; Van Thiel et al., 2020). Gut dysbiosis, or an imbalance in the composition and diversity of the gut microbiota, has been implicated in the pathogenesis of IBS (Carroll et al., 2011). Several studies have reported alterations in the gut microbiota of IBS patients, including reduced microbial diversity, changes in the abundance of specific bacterial taxa, and alterations in the functional capacity of the microbiota (Bhattarai et al., 2017; Carroll et al., 2011).

IBS Dysbiosis

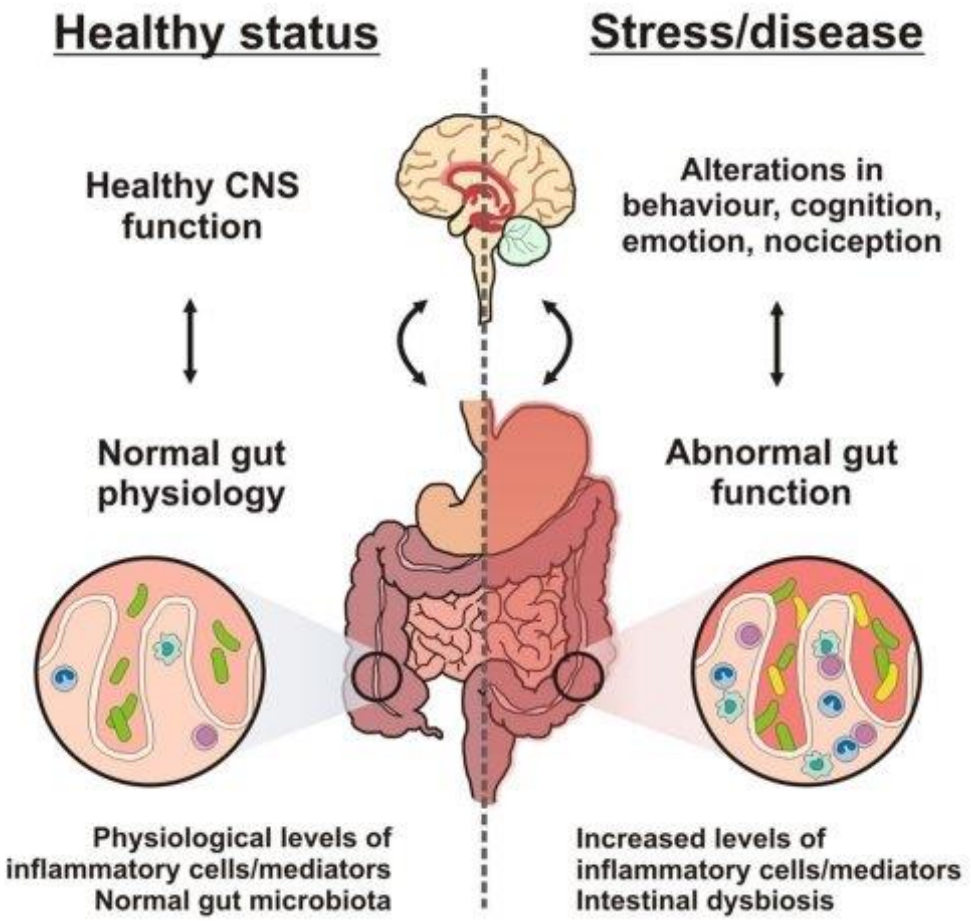
The mechanisms underlying the association between gut dysbiosis and IBS are not fully understood, but several hypotheses have been proposed (Bhattarai et al., 2017). One hypothesis, that is illustrated in Figure 1, is that dysbiosis may alter the gut-brain axis, a bidirectional communication pathway between the gut and the central nervous system that is believed to play a role in the pathogenesis of IBS (Bhattarai et al., 2017; Grenham et al., 2011). Dysbiosis may

affect the composition and function of the gut microbiota, leading to changes in the production of metabolites and neurotransmitters that can affect the activity of the enteric nervous system and the central nervous system (Bhattarai et al., 2017).

Figure 1

Illustration of Microbiota's Effects on the GI Tract and the Brain-gut Axis

Note. This figure shows a model of bidirectional signaling between the brain and gut microbiota in a healthy state versus a chronically stressed state. From "Brain–gut–microbe Communication in Health and Disease," by S. Grenham, G. Clarke, J. F. Cryan, and T. G. Dinan, 2011, *Frontiers in Physiology*, *2*(94), p.6 (https://doi.org/10.3389/fphys.2011.00094). CC BY 4.0.



Another mechanism hypothesis is that dysbiosis may induce low-grade inflammation in the gut, leading to the activation of immune cells and the release of pro-inflammatory cytokines (Bhattarai et al., 2017). Inflammatory mediators may alter the function of the enteric nervous system and the gut-brain axis, leading to visceral hypersensitivity, altered intestinal permeability, and the development of resulting IBS symptoms (Bhattarai et al., 2017). Infiltration of mast cells and macrophages, which are inflammatory cells, within the intestinal mucosa of IBS patients has been noted in some studies as heightened through the heightened expression of toll-like receptors (TLRs) (Bhattarai et al., 2017). It is also known that certain components of bacteria in the gut can act as TLR ligands, such as flagellin and lipopolysaccharide (LPS) (Bhattarai et al., 2017). Increased levels of these bacterial components have been observed to be accompanied by increased TLR expression in IBS patients (Bhattarai et al., 2017).

Several studies have also suggested that dysbiosis may notably affect gut motility and intestinal permeability, via altered serotonergic signaling, which may contribute to the development of IBS symptoms (Bhattarai et al., 2017; Kwon & Khan, 2022). Dysbiosis may alter the expression of genes involved in gut motility and barrier function, leading to changes in the movement of food and waste products through the gut and alterations in the absorption of nutrients (Bhattarai et al., 2017).

The brain-gut axis is also able to influence the abundance of certain bacteria from prolonged depression and psychological stress (Bhattarai et al., 2017; Van Thiel et al., 2020). Stress has recently been shown to shift the gut microbiota composition towards a virulent nature, possibly leading to translocation of bacteria to lymph nodes, which triggers immune responses in the gut (Van Thiel et al., 2020). Stress is also presented as inducing some microbiota alterations (Van Thiel et al., 2020). This includes the increased presence of microbes that induce

inflammation, such as *Helicobacter* and *Streptococcus* gut microbiota, as well as an overall decrease in diversity of the microbiome in the gut (Van Thiel et al., 2020). Stress can alter microbial composition through increased circulatory proinflammatory cytokines, intestinal barrier disruption, and increased HPA axis activity (Bhattarai et al., 2017). Chronic stress and depression have been associated with certain bacteria that are now thought to be implicated in the development of IBS (Bhattarai et al., 2017). *Enterobacteriaceae* family is overrepresented in humans and associated with depression caused by chronic stress (Bhattarai et al., 2017). Psychological stress correlates to reduction in *Lactobacilli spp*. and increase in *Escherichia coli* and *Pseudomonas spp* (Bhattarai et al., 2017). As mentioned, all these bacteria are implicated the progression of IBS (Bhattarai et al., 2017).

Potential Targeted Therapies for IBS

5-HT Signaling as a Target

As a result of the vast amount of knowledge surrounding the involvement of 5-HT in the development and symptoms of IBS, future therapies should focus on bettering the use of 5-HT mediation. Although many of the current therapies for IBS involve administration of 5-HT agonists or antagonists, there is still room for growth in selectivity of these drugs (Del Colle et al., 2020). Manipulation of the pathways controlled by 5-HT signaling continues to be an important target because of the large role played by 5-HT in inflammation, visceral hypersensitivity, permeability, and motility of the GI tract.

Microbiota as a Target

Due to the increasing evidence for gut microbiota involvement on the development of IBS, it has been thought that probiotic formulations could be a good therapeutic strategy for treating IBS (Bhattarai et al., 2017). The studies that have already been conducted in this area

have shown the benefits of probiotic formulas that increase secretion of mucus, protect from harmful bacteria, and reduce pro-inflammatory mediators within the GI tract (Bhattarai et al., 2017). Some studies have shown that probiotics could potentially treat immune dysregulation in IBS (Bhattarai et al., 2017).

Stress as a Target

Chronic stress has been shown to be a prominent factor in the development of many IBS symptoms and pathophysiology. Due to the negative effects physiologically that chronic stress and depression have on the mechanistic pathways related to gut homeostasis, it is imperative to treat psychological stress along with physiologic symptoms of IBS.

Conclusion

Overall, the brain-gut axis influences and modulates the occurrence of IBS through signaling mechanisms heavily influenced by emotional stress. Stress has been shown to increase hypersensitivity and increase or decrease permeability and motility in the gut through heightened or lowered neuro-immune, neuro-hormone, and microbiota-gut crosstalk. A large factor effecting visceral hypersensitivity, abnormal permeability, and microbiota-gut signaling was shown to be low grade inflammation, involved in neuro-immune crosstalk. The other major contributor to phenotypes of pathophysiology was shown to be serotonin signaling. Abnormal 5-HT signaling has been implicated in not only in an observed motility disturbance, but also in visceral hypersensitivity, and permeability alterations in IBS. Understanding the pathophysiology of IBS and how each mechanism could work together provides a greater understanding of potential treatment options so that research can be conducted for them. The largest finding in this research was the influence chronic stress has on every aspect of IBS. Because it can affect each of the potential mechanisms for IBS pathophysiology, stress is an

important aspect of IBS that should most likely not be avoided when considering treatment options. In the case of IBS, psychological treatment could very well lead to a physiological improvement in a patient's life.

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