Chapter

Prebiotics and the Modulation on the Microbiota-GALT-Brain Axis

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Abstract

It is well known that there exists a bi-directional communication system between the enteric nervous system and central nervous system. Recent research has attempted to understand the influence of intestinal bacteria on the brain and behavior. In this manner, it has been observed that pathogenic bacterial products such as lipopolysaccharides (LPSs) can induce behavioral changes such as acute anxiety, depressive symptoms, cognitive deficits, and increased sensitivity to visceral pain. The modulation of LPS production through probiotics, prebiotics, and symbiotics can prevent these changes. In addition to the neuronal, endocrine, and metabolic pathways, it has been observed that the immune mechanism also exerts an influence on the gut-brain axis. The cells of the immune system can undergo phenotypic changes by the induction of certain bacterial species, which can have an important participation in the development of brain disorders. Although the main effect of prebiotics is through the stimulation of probiotic bacteria, in this chapter, we review the indirect therapeutic potential of prebiotics on the brain through the intestinal microbiota, the gut-associated lymphoid tissue (GALT), and other components of the intestinal lumen. Thus, the objective is to elucidate the mechanisms underlying its effects on the gut-brain axis. Here, we will summarize the possible therapeutic effect of prebiotics on intestinal microbiota, the gut-associated lymphoid tissue (GALT), and brain.

Keywords: prebiotics, gut microbiota, central nervous system, enteric nervous system, GALT

1. Introduction

The intestinal microbiota contributes significantly to metabolic, trophic, and protective functions. In this regard, intestinal bacteria are responsible for metabolism of many complex substances into simple components; thus, intestinal microbiota contributes to the digestion of nutrients and has a key role in the nutrition of the host, to the control of certain pathogens and to the improvement of the functions of the local immune system, preventing or participating in some pathologies such as colon cancer [1]. In addition, intestinal bacteria are involved in vitamin synthesis and also in ion absorption [2, 3]. Through their trophic effect, the intestinal bacteria stimulate the proliferation and differentiation of cells on intestinal epithelium [4]; also, these bacteria may contribute to the maturation of immune cells, regulating the proliferation of pathogenic microorganisms and their toxins [1]. In addition

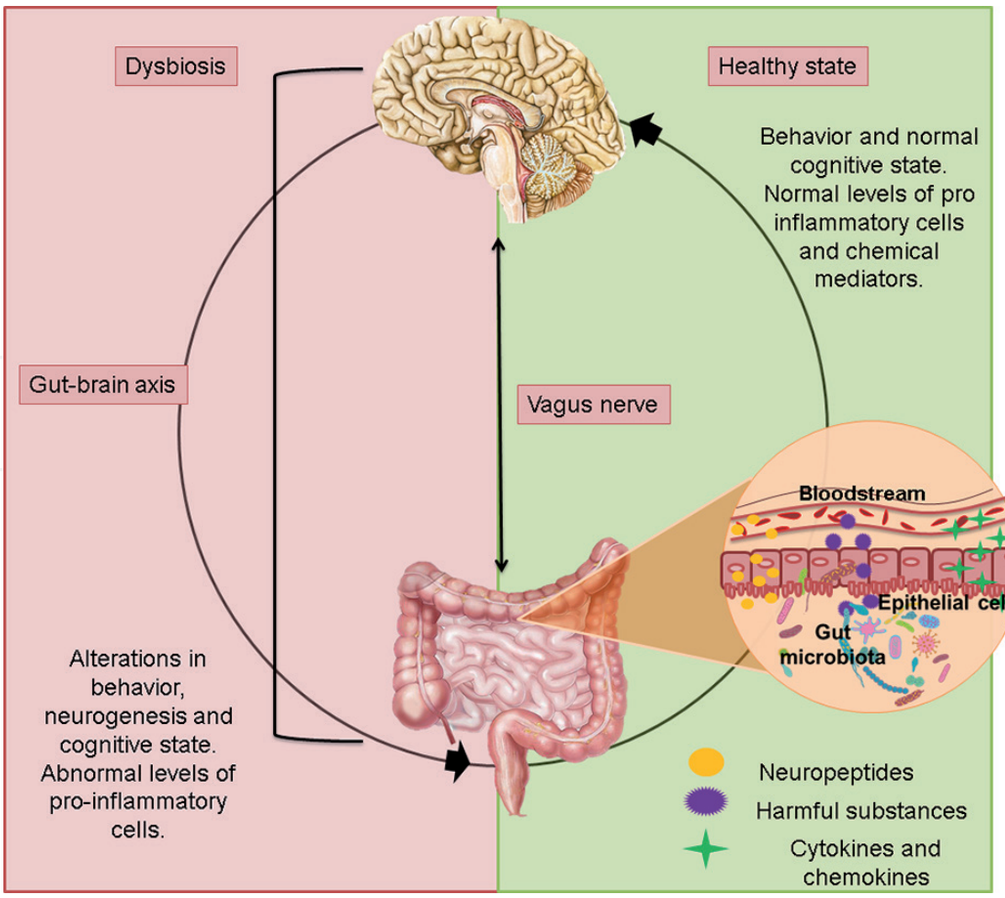

Figure 1.
Microbiota-GALT-brain axis. The vagus nerve makes the connection of the intestine to the brain and vice versa. Dysbiosis causes local alterations in the GALT and in the brain. When there is no dysbiosis, the bacterial metabolites participate in the state of local and systemic health and even more so over the brain.

to the functions above described, it has been proposed that intestinal microbiota exerts indirect functions in other organs such as the liver and brain. Studies performed in humans and in animal models suggest that intestinal dysbiosis has an important role in the development of mood disorders such as depression, anxiety, and Parkinson's disease [5–7]. For these reasons, the interest in exploring the interactions between immune system, intestinal microbiota, and central nervous system (CNS) has increased (**Figure 1**).

On the other hand, when bacterial probiotics are administered in adequate amounts, they confer benefits on host health. The main functions of probiotics are to prevent and ameliorate several digestive and allergic disorders. Also, the microbiota modulates ontogeny and immune system functions, as well as the interactions of the intestine-brain axis to regulate some neurological functions. However, the microbiota effects are not only in intestine but also in peripheral tissues, such as in immune system modulation and interacting with the gut-brain axis to regulate some neurologic functions.

2. The gut-associated lymphoid tissue (GALT)

2.1 Anatomy and physiology

The gut-associated lymphoid tissue (GALT) is a specialized component of mucosal-associated lymphoid tissue (MALT) or mucosal immune system that protects the individual's intestine from invading pathogens. The intestine-associated

tissue extends throughout the small and large intestine, covering an area of 260–300 m^2 approximately. An important function of intestine is nutrient absorption, where an epithelial cells monolayer (also called enterocytes) separates the GALT from lumen and its content. The enterocytes monolayer on luminal surface is coated by a glucocalix layer, which protects them from acidic pH. The intestinal mucosal surface can function as a permeable barrier to the inside of the body. This permeability increases the vulnerability to infections by a variety of infectious agents that invade the human body orally. Therefore, the largest populations of plasma cells that produce antibodies are enriched on GALT, generating a local and systemic humoral immune response with high production of immunoglobulin A (IgA), promoting a robust cellular immunity with cytotoxic, regulatory, and memory functions [8]. GALT can be divided into: (a) inductive sites, composed by lymphoid aggregates or follicles, and (b) effectors sites formed by the lamina propria and the lumen.

In **Figure 2** there is a complete description of GALT, thus GALT forms nodules disseminated into the submucosa and the lamina propria. The largest aggregates form "Peyer's patches," which in the small intestine are located in front of the mesenteric tissue. Locally, new epithelial cells derived from stem cells are constantly produced to regenerate the epithelium. In addition to conventional enterocytes, there are also Paneth cells at the bottom of the epithelial crypts. These cells secrete lysozyme and other antibacterial substances to control the growth of pathogens. These cells are found in the small intestine, especially in the jejunum, and their granules become visible after several hours of fasting. Also, the mucus goblet cells are scattered between other cell types. On the other hand, enteroendocrine cells produce polypeptides and are distributed diffusely throughout the gastrointestinal tract. On the surface of Peyer's patches, "M cells" also called "caveolate cells" are located, which capture antigens and function as intestinal chemoreceptors.

Beneath the epithelial lining is an underlying layer of connective tissue called lamina propria, which is connected to the lymphatic circulation and mesenteric

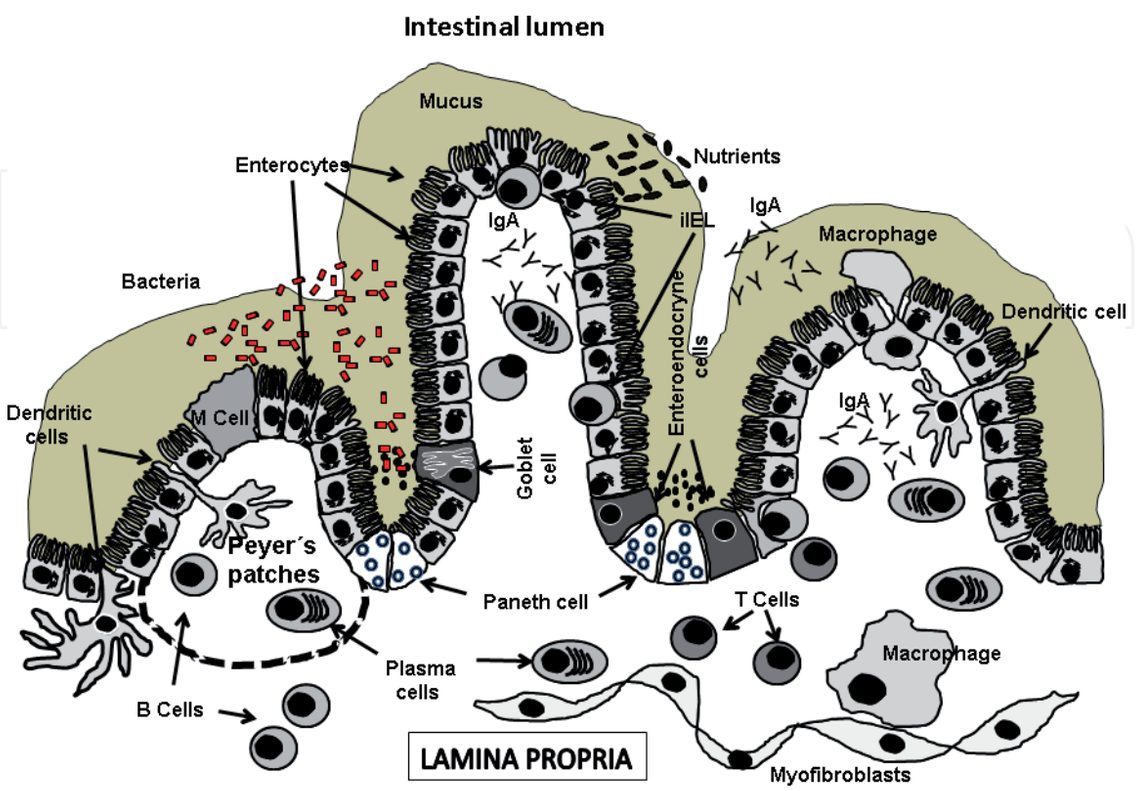

Figure 2.
Anatomy of gut-associated lymphoid tissue (GALT).

lymph nodes. The intestine is constantly exposed to several antigens derived from the diet, microbiota, and a wide variety of bacterial, viral, fungal, and other pathogens. The immunity of this compartment is highly specialized because it is capable of both functions: (a) triggering an immune response and (b) inducing tolerance by suppressing immune response through the interaction of epithelial cells and microbiota [9, 10].

The induction of intestinal mucosa tolerance depends on several factors, such as: (a) nature of antigen (mainly protein antigens) [11]; (b) dose and frequency of antigen exposition [12–14]; (c) kinetics of antigen uptake, because antigenic exposition time with the immune system is key to induce tolerance [15]; and (d) genetic background and age of the host, because there is different susceptibility to infection, depending on age and genetic inheritance. The main antigens reaching GALT are a mixture of free amino acids and short oligopeptides, generated by gastric, pancreatic, or protease action on large proteins, which are absorbed by intestinal epithelial cells [16]. However, some intact proteins or incomplete proteolysis products can reach inductive sites through the following three non-exclusive pathways:

a. The epithelium responds to stimuli from different antigens expressing chemokine and cytokine genes [17]. Consequently, different subsets of cells carrying antigens are recruited to inductive sites [18].

b. M cells, specialized cells in both internalizing and transportation of intestinal antigens [19]. The antigens are endocytosed or phagocytosed, transported in vesicles through the M cells cytoplasm, and released on the basal surface where they are captured by antigen-presenting cells and transported to inductive sites, which will be presented to T and B cells.

c. Lamina propria (LP) dendritic cells (CDs) can go through the epithelial cells to capture antigens directly from the intestinal lumen, preserving the epithelial barrier integrity [20].

It is well known that the intestinal epithelium provides a physical barrier that separates the trillions of commensal bacteria present into intestinal lumen from both underlying lamina propria and deeper intestinal layers. The intestinal epithelium is composed of four cellular subsets derived from a common pluripotent stem cell progenitor: (1) enterocytes which constitute the majority of intestinal epithelial cells (IECs), (2) goblet cells producing mucus, (3) enteroendocrine cells producing hormones, and (4) Paneth cells that produce antimicrobial peptides and lectins. In addition, below the intestinal epithelium, stromal cells, B cells (especially plasma IgA producing cells), T cells, macrophages, and dendritic cells are found in lamina propria. In addition, strategically positioned are intraepithelial lymphocytes or iIELs (specialized T cells) and some dendritic cells, which are located between the IECs to sample the luminal content [20–22]. Thus, intraepithelial lymphocytes are considered the first line of cellular defense against any antigen that enters orally. The iIELs belong to the lymphoid tissue associated to intestine and they are found in a ratio of 1:10 with respect to the epithelial cells along 300 m^2 of intestinal surface [23]. Most iIELs contain abundant cytoplasmic granules for cytotoxic activity; also, effector cytokines such as interferon gamma (IFN-γ), interleukin-2 (IL-2), IL-4, and IL-17 can be secreted [24, 25]. The iIELs are cells that provide an immediate and efficient immune protection to prevent the spread of pathogens. However, to avoid excessive or unnecessary inflammatory responses on intestinal barrier, the iIELs also have regulatory functions [26].

3. Microbial ecosystem of the gastrointestinal tract

The human intestine contains a wide variety of microorganisms, approximately 500–1000 different species, of which the Bacteria, Archaea and Eukarya are the principal ones [1, 27]. The predominant bacteria populations in the intestine are the Bacteroidetes and Firmicutes, constituting about 90% [28]. This diverse ecosystem is called "gut microbiota," which has a symbiotic and mutualism relationship with the host [29, 30]. The intestinal microbiota exerts its own functions and has both direct and indirect influence on host's physiology and health, especially on metabolism. However, several pathologies, including neurological disorders such as irritable bowel syndrome, depression, anxiety, and Parkinson's disease have been associated with alteration of the intestinal microbiota known as "dysbiosis" [5–7].

With respect to dysbiosis, the wide diversity and abundance of gut microbiota population can be modified importantly by host's diet and age, as well as by other factors. The newborn is colonized by bacteria from birth and initially, there are no differences between bacteria population localized on different parts of body. Infants who are born via vaginal delivery are mainly colonized by Lactobacillus and Prevotella, microbial populations closely related to maternal vaginal bacteria populations [31]. In contrast, infants born by cesarean-section (C-section) are exposed to *Staphylococcus* and *Corynebacterium*, which are skin microbes [32]. Thus, vaginal delivery or cesarean section as well as lactation or weaning are important factors that influence intestinal microbiota establishment. On the other hand, initially it was proposed that the prenatal environment is sterile; however, the presence of several bacterial species has been detected on placentas, amniotic fluid, and in meconium of healthy mothers, which suggests that, in the intrauterine stage there is already contact with microbes [33]. The diversity and functionality of the bacterial ecosystem is modified and increased in subsequent years of childhood [33]. Thus, in adult life, the predominant populations are both Bacteroidetes and Firmicutes, while the phylum Actinobacteria, Proteobacteria, Verrucomincrobia, archaea, and eukaryotes decrease importantly [34, 35]. Commonly, intestinal microbiota is very stable in adulthood, although a greater proportion of both Bifidobacteria and Clostridia has been found in the gut of young adults in comparison with older adults [36]. Important changes in composition and function of intestinal microbiota occur on aging. Aging has been associated with changes in intestinal microbiota composition, inducing alterations of multiple physiological functions, including intestine and immune system malfunctioning. An increased proportion of facultative anaerobes bacteria as well as an imbalance of Bacteroidetes/Firmicutes ratio in microbiota are age-related differences. Also, in people over 60 years of age, when the immune system function begins to decline, a significant decrease in Bifidobacteria has been found [37]. These previous findings were also supported by studies performed in intestinal mucosal tissue of aged and young mice, where a reduction in *Akkermansia muciniphila* proportion as well as decrease of antimicrobial factors Ang4 and lysozyme were detected in aged mice. Moreover, an important decrease in genes expression related to immunity was found, including T cell activation and other gene signaling pathways [38].

The high-carbohydrate and high-fat diet composition may produce dysbiosis. It was described that in mice, a Western diet (WD: high-carbohydrate and high-fat diet) intake caused dysbiosis and dysregulated bile acids (BA) synthesis with reduced endogenous ligands for BA receptors, that is, farnesoid X receptor and G-protein-coupled bile acid receptor in the liver and brain [39]. More relevantly, a ketogenic low-carbohydrate high-fat diet induced changes in the oral microbiome of elite endurance athletes; the relative abundances of *Haemophilus*, *Neisseria*, and *Prevotella* spp. were decreased, and the relative abundance of *Streptococcus* spp. was increased [40].

3.1 Functions of the gut microbiota in the host

The main biological functions regulated by the gut microbiota are related to the efficiency to metabolize food and obtain energy. Polysaccharides are the main source of energy in bacterial metabolism, which are transformed into short-chain fatty acids (SCFAs). Bacterial metabolism is not limited only to SCFAs production and obtaining energy. The intestinal microbiota can synthesize several vitamins, aryl hydrocarbon receptor (AHR) ligands on host cells, polyamines [41], folate [42], indole [43], serotonin [44], and other compounds. In addition, intestinal microbiota also produces bacterial toxins called bacteriocins. To date, 13 species of bacteriocins have been found in human feces [45].

The major SCFAs produced by the gut microbiota are acetate, propionate, and butyrate, which are found at 80–130 mM [46, 47]. In this way, the SCFAs represent approximately 70% of the total energy captured by the intestinal epithelial cells. Interestingly, butyrate produced by *Butyrivibrio fibrisolvens* protects against autophagy and energy starvation in the epithelium of gnotobiotic mice [48, 49]. While acetate and propionate have an important role in lipid metabolism, activation of the Gpr43 receptor promotes adipogenesis [50]. Therefore, using antibiotics at subtherapeutic doses, as commonly used in animal production, a dysbiosis is generated by increasing SCFA levels, which consequently induces lipogenesis and hepatic triglycerides synthesis [50].

It is well known that microbiota strongly impacts on the expression of genes and proteins on host intestinal epithelial cells. In axenic mice, it has been found that in intestinal colonization by *Bacteroides thetaiotaomicron,* an important gene expression was induced. Expression of these genes is involved in protection, intestinal barrier function regulation, epithelium vascularization, and digestion/absorption of nutrients by increasing amino acid metabolism [50, 51]. It also participates in the regulation of endocrine, neurological, and bone density functions [33], as well as in the metabolism and absorption of phytochemicals such as polyphenols and drugs [50].

Additionally, several studies have shown that microbiota has an important role in peripheral and intestinal immune system ontogeny, as well as intestinal epithelium renewal [52, 53]. Also, microbiota-epithelial cell interaction indirectly controls the expenditure and storage of energy in the host [54]. Dysbiosis has been associated with a several pathologies affecting directly the digestive tract, including chronic inflammatory bowel diseases, colorectal cancer, constipation, and diarrhea; but in peripheral organs, it can also induce allergies, arthritis, or neurological disorders [55]. In this way, correcting the dysbiosis could improve the symptoms of diseases like irritable bowel syndrome (IBS) and functional diarrhea [56].

Intestinal bacteria are importantly involved in development and regulation of the immune system [57–59]. On this regard, mice grown under germ-free conditions exhibited several abnormalities, including hypoplastic Peyer's patches, IgA-producing cells reduction, relatively poorly structured spleen and lymph nodes, and decrease in proportion of Treg cells in colon [53]. Interestingly, when mice were exposed to intestinal bacteria for several weeks, the structure and function of immune system cells were restored [60]. Moreover, it has been shown that lipopolysaccharides from gram negative bacteria, as well as peptidoglycans from gram positive bacteria, activate Toll-like receptors (TLRs), inducing different immune responses [61]. Also, the expression of angiogenin 4 by *Bacteroides thetaiotaomicron* is induced. Angiogenin 4 is an important immune response regulator with microbicidal activity against a wide range of intestinal microbes, including bacterial and fungal pathogens [62]. A zwitterionic capsular polysaccharide of *Bacteroides fragilis* is an antigen related to T CD4 + effector cells function [63]; it also protects mice from *Helicobacter hepaticus* infections by suppressing IL-17 production and other immunological mechanisms [64]. Moreover, the genus Bifidobacteria is a producer

of acetate that inhibits the translocation of Shiga toxin from *E.coli* 0157 suppressing colon inflammation [65]. In addition, *Bacteroides*, *Turicibacter*, and *Barnesiella* bacteria strains interact with T CD8 + cytotoxic cells in the mucosal compartment of both the small intestine and the colon [66]. Finally, the gut microbiota also regulates the interaction of dendritic cells with regulatory T cell through TLR 2 signaling, which induces an increased susceptibility to chronic inflammatory diseases such as colitis [67].

3.2 Host's immunomodulatory activity on the gut microbiota

The interactions between environmental signals and intestinal immune system are necessary to maintain a stable equilibrium and regulate the protective function of the intestinal barrier. Thus, in order to prevent microbial colonization and transepithelial migration, several chemical substances are produced by the intestinal epithelial cells, such as gastric acid, enzymes (lactoferrin, lysozyme), antimicrobial peptides (defensins), mucins, and nitric oxide [68]. On the other hand, signaling through Toll-like receptors (TLRs) is very important for the activation of innate immune system. TLRs recognize a wide range of common antigens present in pathogens, activating the adaptive immune system for the generation of multiple highly specific and immunocompetent clones [69].

4. Connection between enteric nervous system (ENS) and central nervous system (CNS)

The enteric nervous system (ENS) extends from the esophagus to the anal region. The main functions are: (1) stimulation of glandular secretions, (2) motor functions such as peristalsis, and (3) ions and water exchange. The neurons found in CNS are subdivided into two main plexuses: the myenteric plexus and the submucosal plexus [70]. The former is responsible for peristalsis and second regulates the glandular secretions and control of blood flow. There are extrinsic fibers connecting these two plexuses, which are stimulated by both sympathetic and parasympathetic nervous system, communicating directly to spinal cord, part of the vagus nerve, and pre-vertebral ganglia of the sympathetic nervous system, although the ENS is able to function independently [71].

Generally, there are different types and subtypes of neurons throughout the ENS: excitatory neurons of the intestine, secretomotor, vasodilator, and non-vasodilator; some of them innervate whole endocrine cells and others intrinsic visceral afferent neurons. Most of the different types of neurons participate in reflexes corresponding to each plexus (myenteric and submucosal) (**Figure 3**).

The neurotransmitters secreted by enteric neurons are varied, the acetylcholine (excitatory effect) and noradrenaline (inhibitory effect) being the most studied [72]. In addition, the communication of the ENS with the CNS is not only through the secretion of these neurotransmitters. Several studies have shown that bacterial metabolites generated in the intestinal ecosystem have a direct impact on the brain. Thus, it is well known that the intestinal microbiota has an important effect on CNS, because the homeostasis and intestinal functions can be regulated by the CNS [73]. The CNS and intestine connection may occur through several pathways, including: neuronal, hormonal, immune system, and intestinal bacterial metabolites [74]. Regarding the neuronal connection, the intestine is directly connected to the brain through the vagus nerve; thus, intestinal microbiota may stimulate the enteric nervous system [75]. In addition, several reports have shown that a defective communication between the brain and intestine microbiota is associated to anxiety, depression, inflammatory bowel disease, and other diseases

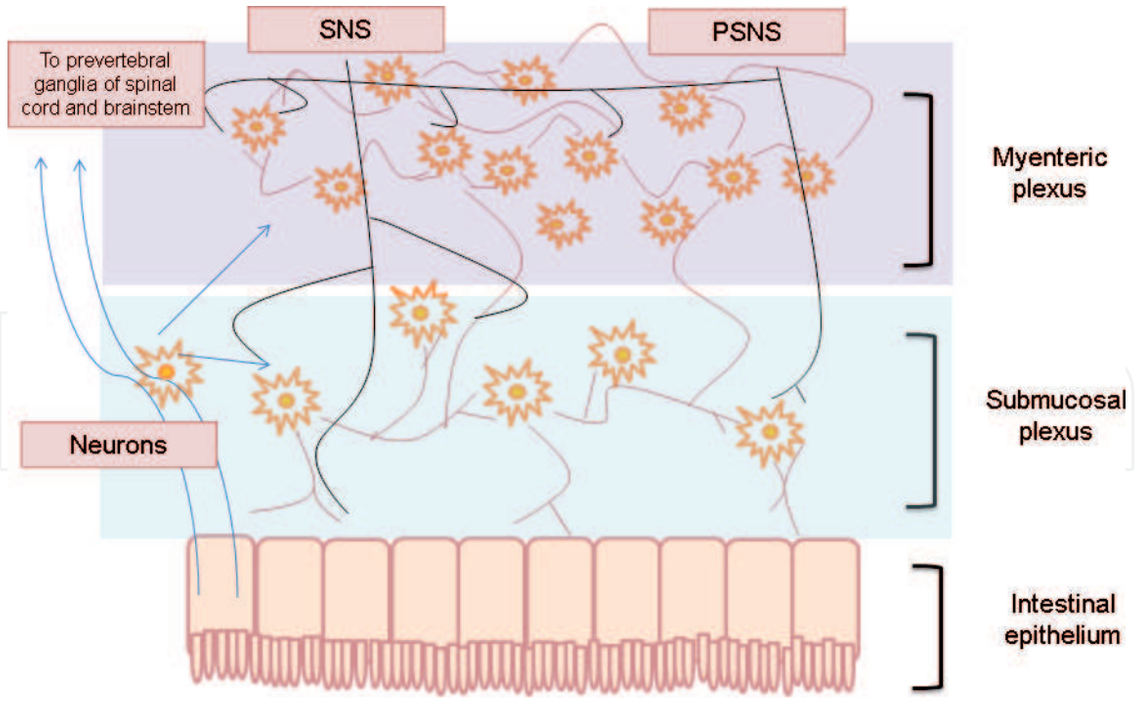

Figure 3.
Structure of the enteric nervous system. Both plexuses can be identified (myenteric and submucosal). The extension goes from the intestinal wall to the enteric plexuses and from there to the pre-vertebral ganglia of the spinal cord and brainstem. SNS, sympathetic nervous system; PSNS, parasympathetic nervous system.

[76–78]. Interestingly, behavior changes in elderly have been associated with decreased immune function, resulting in alteration of intestinal microbiota-brain connection [79].

4.1 Dysbiosis and its effect on the neuroplasticity and behavior

In the first weeks of life, microbiota diversifies into a microbial community in which anaerobic microorganisms predominate [80]. This early colonization coincides with hypothalamic-pituitary-adrenal (HPA) axis activation, which has an important role in the innervation of the gastrointestinal (GI) tract and enteric nervous system (ENS) function. Likewise, the production of 5HT by enterochromaffin cells is regulated by the intestinal microbiota, inducing de *novo* synthesis of 5HT [44].

Several reports have shown the relation between intestine and the CNS through metabolic, neuroendocrine, and immunological pathways, impacting neuronal plasticity and cognition. Production of proinflammatory cytokines (IL-1) in intestinal lumen may affect the brain through vagus nerve [81]. Also, bacterial products like lipopolysaccharide (LPS) can increase cytokines production, as well as induce both neuroinflammation and neurodegeneration [82]. In addition, it was shown that WD-fed mice had intestinal dysbiosis, which was accompanied by inflammatory signaling in the brain, microglial activation, and reduced neuroplasticity [83]. Therefore, dysbiosis of gut microbiota may increase the cytokines production and neuroinflammation, affecting mood, or it could induce psychiatric disorders such as depression and anxiety as was described in animal models [56] as well as in comparative studies performed in humans [5, 84]. Moreover, in maternal immune activation (MIA) mouse model, intestinal dysbiosis induced both higher production of both 4-ethylphenylsulfate (4EPS) and indolepyruvate, leading to autism spectrum disorder (ASD). Interestingly, with *Bacteroides fragilis* administration, these behavioral symptoms were ameliorated [85].

A mechanism by producing substrates from bacterial metabolism such as SCFAs from the fermentation of dietary fiber has been described. The SCFAs regulate the metabolism of glucose and cholesterol [86]. Most importantly, the SCFAs (acetate, propionate, and butyrate) treatment alleviated the psychosocial stress-induced alterations in mice; the effect observed was selective, because the stress-induced body weight gain, fecal SCFAs, and the colonic gene expression of the SCFAs receptors free fatty acid receptors 2 and 3 remained unaffected by SCFAs supplementation [87].

Other studies also have shown that dysbiosis may induce mood alterations. For example, dysbiosis induced by antibiotics treatment in mice leads to cognitive and behavioral alterations as well as to neurological changes [88, 89]. In addition, in juvenile mice with dysbiosis, after a 2-week antibiotic treatment, the levels of mRNA and protein of brain-derived neurotrophic factor (BDNF) and tropomyosin-related receptor kinase B (TrKB) in hippocampus CA3 and dentate gyrus subregions, respectively, significantly increased [89]. Most importantly, patients with disorders like depression, anxiety, and eating disorder psychopathology have a significantly lower microbial alpha diversity as compared with healthy subjects [5]. Interestingly, depression and anxiety symptoms may be improved by fecal microbiota transplantation in patients with irritable bowel syndrome (IBS), functional diarrhea (FDR), or functional constipation (FC). Thus, the increase of microbiota diversity may improve the patient's mood [56].

5. Prebiotics

Since its initial description in 1995, the concept of prebiotic has been in constant evolution. Currently, according to Gibson and Roberfroid, a probiotic has been defined as "any substance present in diet, which specifically stimulates the growth and/or the fermentative activity of one or a limited number of bacteria species of intestinal microbiota, generating beneficial effects on health of host as a consequence of changes on either bacterial composition or metabolic activity" [90].

Generally, a food ingredient is considered as a prebiotic when it has the following characteristics: (a) it must be kept in good condition until reaching the distal portions of the intestine; that is, it is not absorbed in the anterior part of the gastrointestinal tract and resists the hydrolysis of digestive enzymes [91] such as α-glucosidase, maltase, isomaltase, and sucrase [92]; (b) it must act as a selective substrate in the growth and/or metabolism of one or a limited number of beneficial bacterial species, such as Lactobacillus spp. and Bifidobacterium spp.; and finally (c) it must positively stimulate the microbiota, by increasing beneficial microorganisms and reducing pathogenic bacteria [93].

It is well known that proliferation of bifidobacteria and lactobacilli is favored by prebiotics; moreover, the proliferation of bacterial pathogenic strains such as *Clostridium*, *Escherichia*, *Campylobacter*, *Enterobacterium,* or *Salmonella* is inhibited. It has been proposed that intestinal microbiota is in involved in inhibition prebiotics mechanisms, by either competition for adhesion sites to the mucosa, or changes in the intestinal environment, such as (a) a reduction in pH as result of the synthesis of SCFA and (b) production of metabolites inhibiting pathogens proliferation, such as bacteriocins [94].

5.1 Types and sources

As already mentioned, prebiotics are normally ingested in the diet; however, only some carbohydrates (poly and oligosaccharides), whose chemical structure has β-type bonds, some peptides, some proteins, and certain lipids such as esters and ethers are food ingredients qualified as prebiotics [95].

Different types of oligosaccharides considered as prebiotics have been reported, among them are: fructooligosaccharides (FOS), oligofructose (OF), the inulin type fructans (ITFs), galactooligosaccharides (GOS), transgalactooligosaccharides (TOS), and lactulose. However, there are others less known such as isomaltooligosaccharides (IMOS). Xylooligosaccharides (XOS) and mananooligosaccharides (MOS) also have a probiotic potential [90, 95]. It is well known that sucrose and starch are the main carbohydrates found in higher plants, followed by glucomannans and fructans are the main reserve sources in the vegetable kingdom [96, 97].

5.2 Interaction of prebiotics in gut microbiota

The non-digestible carbohydrates or prebiotics are selectively fermented by the microbiota that produces important metabolites for the health of the host, the SCFAs acetate, propionate, and butyrate mainly. Likewise, prebiotics have a direct impact on the gut-associated lymphoid tissue (GALT) with immunomodulatory functions [98]. The symbiotic association between the host and the microbiota is fundamental in its physiology. The increase of the beneficial populations in the intestinal lumen affects the establishment of opportunistic pathogens, contributing to the strengthening of the GALT.

5.3 Potential effects on microbiota-GALT-brain axis

The stimulation of probiotic bacteria by prebiotics contributes to the increase of the production of beneficial metabolites for the host, such as the SCFAs. However, prebiotics not only have an impact on the intestinal microbiota. Oligofructose, an inulin type fructan, can bind to cellular receptors of pathogenic bacteria and block adhesion to the surface of enterocytes, helping to prevent colonization. In the same way, β (2→1)-fructans are ligands of TLR2, TLR4, TLR5, TLR7, TLR8, and NOD2. Moreover, levan (2→6)-fructans appears to be a recognized of TLR4 to reduce IgE serum levels and Th2 responses [98]. Bacterial metabolites travel through the vagus nerve to reach specific brain regions such as cerebellum and hippocampus and modify gene expression [99]. It has been described that alterations in intestinal microbiota due to exposure to chronic stress are reversed with the administration of prebiotics and/or probiotics [100].

According to the classification of microbiota obtained by statistical analysis of sequence data, an alpha diversity has been identified, which describes the diversity of bacteria within a single individual; while beta diversity describes the diversity of specimens between different individuals. It has been shown that different types of prebiotics have an effect on the microbiota-GALT-brain axis in mice and human models. Prebiotics treatments (isolated from acorn and sago) induced increased β-diversity in heart failure patient's fecal microbiome, while no significant change in β-diversity was seen in healthy fecal microbiome. Alpha diversity was significantly higher in both healthy and diseased fecal microbiome, which was accompanied with an increase of the beneficial bacteria and SCFAs. Moreover, prebiotics treatment ameliorated HFD-induced glucose intolerance and insulin resistance in diabetic mice. Feeding both prebiotics treatments and inulin increased SCFAs levels in the mouse gut, and decreased the gut hyperpermeability and mucosal inflammatory markers in HFD-fed mice. The expression of pro-opiomelanocortin was also modulated by prebiotics administration, suggesting an important role in the hypothalamic energy signaling in the mice [101]. In diabetic db/db mice, the administration of oligofructose increases the expression of tight junction proteins occludin and ZO-1, which improves the integrity of the BBB in the hypothalamus and normalizes

the expression of mRNA of IL-6 in the hippocampus; however, it does not improve alterations in behavior or in neurogenesis [102].

Moreover, the administration of prebiotic chitosan oligosaccharides (COSs) in Male Sprague-Dawley rats increases cognitive function and reduces levels of TNF-α and IL-1β, both pro-inflammatory cytokines [103]. In a mouse model of amyotrophic lateral sclerosis, the oral administration of GOS reduced the motor neuron death and muscular atrophy and increased the levels of serum folate, vitamin B12, and homocysteine [104]. In the same way, in a mouse model with lipopolysaccharide-induced anxiety, the administration of bimuno-galacto-oligosaccharides (B-GOSs) reduced the pro-inflammatory cytokine IL-1β and expression of cortical 5-HT2A receptors [78]. In a mouse model of vascular dementia, the β-glucan from barley and arabinoxylan from the yeast *Triticum aestivum* demonstrated a protective effect [105].

Interestingly, our research group previously demonstrated that the administration of oligofructose and *Agave* fructans decreased TBARS levels and carbonyls in learning and memory regions of the brain of overweight mice [106]. Also, in high-fat diet-induced obese mice, prebiotics not only reduces the oxidative damage in the same regions but also increases the levels of BDNF and GDNF [107].

On the other hand, the administration of synbiotics (probiotics and prebiotics in combination) shows interesting effects. In infants with cow's milk allergy, the treatment with based formula of amino-acid and symbiotics (a combination of fructo-oligosaccharides and *Bifdobacterium breve* M-16V) increased *Bifidobacterium* spp. and *Veillonella sp* bacteria substantially. Additionally, the lactate levels were increased, but the valerate and SCFAs levels were decreased [108]. Prolonged consumption of ADR-159 diet (fermentate generated from *Lactobacillus fermentum* and *Lactobacillus delbrueckii*) had no effect on anthropometrics or general health, but mice fed with ADR-159 presented increased sociability and lower baseline corticosterone levels (stress hormone). The diet also induced significant changes in the microbiota [109].

In patients with major depressive disorder (MDD), probiotics treatment ($\geq 10 \times 10^9$ CFU of freeze-dried *Helveticus* R0052 and *B. longum* R0175 bacteria) for 8 weeks improved depression symptoms, but the serum inflammatory cytokines marker (TNF-α, IL-1β, IL-6, and IL-10) levels were not improved, and the urinary cortisol levels decreased by 20% of baseline. However, the prebiotics treatment (galactooligosaccharides, GOS) had no effect on depression symptoms nor inflammatory marker levels [110]. In a like manner, oral GOS administration reduced the human stress hormone cortisol and increased attentional vigilance to positive versus negative stimuli [111].

6. Conclusions

The effect of the probiotics on the intestinal microbiota is quite important not only for the functions on intestine, but also for the development and function of immune system, metabolism, and central nervous system. Moreover, these systems are closely related, so that any alteration will impact their functionality. Therefore, the balance on microbiota-gut-central nervous system axis is very important to maintain the adequate functions of these systems.

Intestinal dysbiosis leads to alterations in development and function of central nervous system, which is significantly improved upon intestinal colonization with normal microbiota and probiotics treatment. To date, the interaction of microbiota-intestine-brain axis is not completely clear. For this reason, it is interesting to redirect the investigation of CNS diseases whose pathological mechanism is unknown.

Acknowledgements

We are thankful to the University of Guanajuato for their financial support (220/2019) to J R-E and 191/2019 to SLB. Cristina D. Balcón thanks CONACYT for the scholarship with support number 712500.

Conflict of interest

The authors declare no conflict of interest.

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