Review Article

Gut Microbiota-brain Axis

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Abstract

Objective: To systematically review the updated information about the gut microbiota-brain axis.
Data Sources: All articles about gut microbiota-brain axis published up to July 18, 2016, were identified through a literature search on PubMed, ScienceDirect, and Web of Science, with the keywords of "gut microbiota", "gut-brain axis", and "neuroscience".
Study Selection: All relevant articles on gut microbiota and gut-brain axis were included and carefully reviewed, with no limitation of study design.
Results: It is well-recognized that gut microbiota affects the brain's physiological, behavioral, and cognitive functions although its precise mechanism has not yet been fully understood. Gut microbiota-brain axis may include gut microbiota and their metabolic products, enteric nervous system, sympathetic and parasympathetic branches within the autonomic nervous system, neural-immune system, neuroendocrine system, and central nervous system. Moreover, there may be five communication routes between gut microbiota and brain, including the gut-brain's neural network, neuroendocrine-hypothalamic-pituitary-adrenal axis, gut immune system, some neurotransmitters and neural regulators synthesized by gut bacteria, and barrier paths including intestinal mucosal barrier and blood-brain barrier. The microbiome is used to define the composition and functional characteristics of gut microbiota, and metagenomics is an appropriate technique to characterize gut microbiota.
Conclusions: Gut microbiota-brain axis refers to a bidirectional information network between the gut microbiota and the brain, which may provide a new way to protect the brain in the near future.

Key words: Gut Microbiota; Gut Microbiota-brain Axis; Metagenomics; Microbiome

Introduction

The latest research showed that changes in gut microbiota could affect the brain's physiological, behavioral, and cognitive functions.[1-5] In 2013, the United States launched a special research project on gut microbiota-brain axis. Since then, this field, especially the interaction between gut microbiota and the brain, has gradually become the focus of neuroscience.[2-4] Although the exact mechanism of gut microbiota-brain axis has not yet been fully understood and clarified, the evidence from animals and human studies has showed that gut microbiota can play an important role in brain behavior and cognitive development by producing hormones, immune factors, and metabolites, which also indicated that altering the gut microbiota may improve or even cure brain diseases.[6-14]

The gut microbiota and the brain interact with each other, and the gut microbiota can be regarded as an independent variable in gut microbiota-brain axis, its effect on the brain regarded as a dependent variable. Therefore, this article focused on the influence of gut microbiota on the brain and described the latest progress in gut microbiota, cognitive process of influence of gut microbiota on the brain in neuroscience domain, and the possible measurement and detection of gut microbiota.

Gut Microbiota

Gut microbiota is a complex community that helps to maintain dynamic metabolic ecological balance.[15] There are an estimated 100 trillion bacteria in an adult's body, 80% of which exist in the gut, about ten times as many

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as cells in human body. The gut microbiome host more than 100 bacterial species that encode 150 times as many genes as the human genome.[16,17] It is well-recognized that human microbiome is composed of more than 5000 strains of microbes and greater than 1000 kinds of microflora.[16,17] The bacteria, mainly anaerobic bacteria, dominate this environment, and others including virus, protozoa, archaea, and fungi also involve in this environment.[18,19] The microbiome is mainly defined by two bacterial phylotypes, *Bacteroidetes* and *Firmicutes*, and the amounts of *Proteobacteria*, *Actinomyces*, *Fusobacterium*, and *Verrucomicrobia* are relatively small.[20]

Gut microbiota is changing with human development and influenced by various stress factors. Babies receive the initial microbiome from their mothers.[20,21] After 1-year-old, the infants form a complex gut microbiome like adults.[22,23] The compositions of gut microbiota are not fixed, and change with increasing age. The dynamic changes are markedly different among different individuals, but the common effect is a macrobalance.[24] The changes of beneficial bacteria can significantly affect the health of individuals, while some factors such as infection, drug, illness, and diet may change the microbiome.[25-27]

Some evidence showed that stress in the first few years of life could lead to the change of microbiota, and this change is a risk factor for stress-related disorders in adulthood.[28,29] Maternal separation could lead to the reducing number of *Lactobacillus* in the feces of baby rats three days later, which had a long-term effect on the gut microbiome.[28] Prenatal stress changed the compositions of microbiota by reducing the total number of *Bifidobacterium* and *Lactobacillus* in the rhesus monkey.[30] Moreover, prenatal antibiotic treatment increased offspring's susceptibility to experimental colitis.[31] These results indicated that stress could change the gut microbiome.

Gut microbiota has multiple functions. First, gut microbiota constitutes the intestinal barrier, promotes the continuous existence of gut microbiota, stimulates intestinal epithelial cell regeneration, and produces mucus and nourishes mucosa by producing short-chain fatty acids (SCFAs).[32] Gut microbiota is involved in the maturation of immune system by stimulating innate immune system in the early stage of life, which leads to the maturity of intestinal-related lymphoid tissue, inspires the acquired immunity by stimulating local and systemic immune responses,[33] intestinal synthesis and metabolism of certain nutrients, hormones and vitamins, and plays an important role in drug and poison removal. Under physiological conditions, gut microbiota continues to stimulate the immune system, leading to a state of "low degree of physiological inflammation", which is a rapid and effective mechanism for defending against pathogens.[34] In addition, bacterial colony also plays a role of protective competition in gut, producing nutrition for the survival of pathogen and cytokines that can inhibit the growth of the microorganism.[35]

Influence of Gut Microbiota on Cognitive Process of Brain

The effect of gut microbiota on the brain was seldom recognized by people at first, except the pathogenic microorganism in the gut can pass through the blood-brain barrier and affect the brain, for instance, rabies virus could elicit aggression, agitation, and a fear of water when it enters the brain.[3] However, a public health emergency aroused people's attention to the possible relationship between the gut microbiota and the brain. In the year 2000, the flood occurred in the town of Walkerton, Canada, making the drinking water polluted by *Escherichia coli* and *Campylobacter jejuni*. Among the 4561 infected participants, 2451 of them completed a reassessment 8 years later, and 1166 of them were diagnosed with irritable bowel syndrome (IBS). Among these IBS patients, anxiety and depression were found to be independent risk factors for continuous IBS.[13] But at that time, the interaction between gut microbiota and the brain had not been taken seriously by neuroscientists.

In the year 2011, a study by Diaz Heijtz *et al*.[5] showed that compared with conventional mice who were growing in specific-pathogen-free (SPF) environment, germ-free (GF) mice under the experimental conditions had less anxiety-like behaviors and increased 5-HT synthesis in the thalamus. When moving the adult GF mice to SPF environment, its reduced anxiety-like behavior did not increase, but its offspring's anxiety behavior returned to the normal state, which indicated that there was a critical time window for the influence of gut microbiota on behavior development. At that time, although there were an increasing number of related studies, most of them were conducted by gastrointestinal scientists alone, few were performed by gastrointestinal scientists together with psychologists. The focuses of the studies were peripheral and behavioral variations rather than variation in the brain. However, the study of Diaz Heijtz *et al*. sparked an interest of scientists in this field, and they hoped to directly study the underlying mechanism about the role of gut microbiota in the brain.

In the year 2013, the National Institute of Mental Health (NIMH) launched a special project on exploring the mechanism involved in gut microbiota-brain communication, with a view to develop new medications or noninvasive treatments for mental diseases. Since then, studies on the influence of gut microbiota on the brain have been increasing, and gut microbiota-brain axis has become one of the focuses of neuroscience. The core of the axis was the interaction between the gut microbiota and the brain.[2-4] Gut microbiota has an important influence on the brain through the neural network, neuroendocrine system, and immune system.[2-4,6,7]

During 2014 and 2015, NIMH offered a special fund of 1 million US dollars to study the gut microbiota-brain axis. In the year 2015, the United States Navy Institute planned to provide a special fund of 14.5 million US dollars in the next 6–7 years to research the role of the gut in cognitive and

stress disorders. The European Union has launched a 5-year MyNewGut project (10.1 million US dollars) for research on brain development and related disorders.[3]

How Does Gut Microbiota Affect the Brain

Currently, the exact mechanism of communication between the gut microbiota and the brain has not yet been fully understood and clarified. Generally speaking, gut microbiota exerts effects on the brain not only through the nervous system (gut-brain's neuroanatomical pathway) but also through the endocrine system, immune system, and metabolic system. A bidirectional communication between the gut and the brain is referred to as the gut-brain axis.[36,37] Interaction of gut microbiota and gut-brain axis is referred to as the gut microbiota-gut-brain axis (hereinafter referred to as the gut microbiota-brain axis).[38,39] In the gut microbiota-brain axis, because gut microbiota can be used as an independent variable and changed intentionally, more emphases are placed on the role of microbes in gut microbiota-brain axis.[40]

Neuroanatomical pathways

The gut can interact with the brain through two neuroanatomical pathways. The one is mutual information exchange directly between gut and brain by the autonomic nervous system (ANS) and vagus nerve (VN) in the spinal cord; another one is a bidirectional communication between gut and brain through the bi-communication between enteric nervous system (ENS) in the gut and ANS and VN within the spinal cord. The neural anatomical pathways for controlling gut functions form a hierarchic four-level integrative organization:[41,42] the first level is the ENS, including myenteric ganglia, submucous ganglion, and gut glial cells;[43,44] the second level is prevertebral ganglia regulating peripheral visceral reflex responses;[45] The third level is the ANS in the spinal cord (from T5-L2 sympathetic nerve and S2-S4 parasympathetic nervous system) and brain stem nucleus tractus solitarius and dorsal motor nucleus of VN, which receive and give the origin of afferent and efferent fiber of VN, respectively. The most important effect of the dorsal motor nucleus of VN is prominent in the upper gastrointestinal tract, and the cholinergic neurons on myenteron of upper gastrointestinal tract regulate vagal excitability effect;[46] and the fourth level is the higher brain centers. Information from cortex and subcortical centers including basal ganglia and funnels down to peculiar brainstem nuclei. Brainstem nuclei control many gut functions. The afferent fiber of VN stops at the brain stem nucleus tractus solitarius, which then gives fiber upward and arrives at thalamus, lobus limbicus, and insular cortex through parabrachial nucleus. Spinal afferent fiber goes upward within spinothalamic tract and spinal tract to the thalamus (spinothalamic tract) and gracile nucleus and cuneate nucleus of medulla oblongata (spinal tract), respectively, then project fiber to thalamus through lemniscus medialis. Fiber is gave from thalamus and projected to the primary sensorimotor areas and insular cortex. Damages and abnormalities at the above-mentioned levels can influence the regulation of intestinal function, including local intestinal reflexes, and external neural control.[42]

Direct neural communication between gut microbiota and the brain is mainly realized through VN, i.e., bacteria stimulates afferent neurons of ENS,[47] and the vagal signal from the gut can stimulate the anti-inflammatory response, preventing against pyosepticemia caused by microorganisms. Further research showed that many effects of gut microbiota or potential probiotics on brain functions were independent on vagal activation,[39,48] and bacteria settled in the gut played a critically important role in individual's postnatal development and the maturation of the immune system, the endocrine system, and the nervous system.[47]

Neuroendocrine-hypothalamic-pituitary-adrenal axis

Gut microbiota is helpful to the maturation of neuroendocrine. Lacking gut microbiota and low/lack of expression of toll-like receptors (TLRs) contribute to producing a neuroendocrine response to the pathogen in the gut.[49,50] For example, the response of TLR4-knockout mouse to lipopolysaccharide (LPS) produced by Gram-negative bacteria was reduced.[51] Griseofulvin (GF) mouse is one of the most appropriate models to study the hypothalamic-pituitary-adrenal (HPA) axis regulated by the microorganism. Compared with SPF mice, mild restraint stress led to significantly elevated corticosterone and adrenocorticotropic hormone in GF mice. GF mouse's stress response could be partially reversed by fecal microbial transplant, and completely reversed over time by single *Bifidobacterium infantis*.[52] The study clearly showed the feces containing gut microbiota were vital for the postnatal development of appropriate stress reaction, and the timing that microbiota appeared in early life was a very narrow window, which was extremely important for normal development of HPA axis.

Gut microbiota can affect neural circuits and behavior related with the stress response. Compared with SPF mice, GF male mice had the reduced brain-derived neurotrophic factor (BDNF) and 2A subtype of N-methyl-D-aspartic acid receptor (NMDA receptor) expressions in cortex and hippocampus.[52] Neufeld *et al*.[53] have found that mRNA of BDNF in the hippocampal area was improved in female mice, which conflicted with the results reported by the earlier studies. Clarke *et al*.[54] have also found that mRNA of hippocampus BDNF in male mice was reduced and the 5-HT functional system was significantly changed, but these changes could not be found in female mice. These findings suggested that the regulation of gut microbiota-brain axis may be dependent on gender. Many studies have shown the changes of hippocampal NMDA and 5-HT1A receptor of GF animals.[52] These receptors affect the release and expression of the corticotropin-releasing hormone of the hypothalamus and then change the function of HPA.

Stress and HPA axis can affect the composition of the gut microbiome. Early stress and maternal separation could lead to a long-term change of HPA, and also had a long effect on

the microbiome.[55,56] When compared with rats nonseparated from the mother, the diversity of 16S ribosomal RNA in adult rats, who received mother separation for 3 h/day from day 2 to day 12 after birth, revealed that stress significantly changed microbiome in feces.[28] Microbiome composition in mouse exposed to a long-term restraint stress was significantly different from that of a nonstressed mouse.[57] Recently, using the above method and the repeated social interaction, stress can reduce the quantity of *Bacteroides* at cecum and increase the number of *Clostridium*.[58] Stress also increased interleukin-6 and monocyte chemotactic protein 1 (MCP-1) levels in blood, and MCP-1 was significantly related with the changes of three kinds of stress-induced bacteria of *Enterococcus faecalis*, *Pseudobutyrivibrio*, and aerogenic bacteria Dorea strain.

Gut immune system

Development of gut immune system depends on gut microbiota.[59,60] GF mice almost had no immune activity, but they could generate immune function when giving certain microbiota.[61] For example, the segmented filamentous bacterium in the gut can restore the full functions of gut B and T lymphocytes.[62-64] Bacteria communicate with the host through a variety of ways, and the receptors-TLRs of host cell play a key role in the communication between bacteria and host. There are ten kinds of TLRs in the human innate immune system, which have been identified as pattern recognition receptors.[65] These receptors are a part of the innate immune system, which is the first step to produce cytokine response and is also widely distributed on neurons.[66] Hence, neurons also respond to bacterial and viral components. Intestinal epithelial cells can transport microbial composition or metabolites into the inner environment, and the nervous system also interacts with these bacterial and viral components.[67] The balance of gut microbiota may change the regulation of inflammatory response, and this mechanism may also get involved in the regulation of emotion and behavior.[41,61,68-70]

Neurotransmitters and neural regulators synthesized by intestinal bacteria

Gut bacteria can synthesize gamma amino acid, butyric acid, 5-HT, dopamine, and SCFAs,[48,71] and these substances can exchange between cells of microorganism,[48] especially intestinal cells in the gut can produce many 5-HT that have an effect on the brain. Bacterial enzymes can also produce neurotoxin products such as D-lactic acid and ammonia.[3,72] Hence, a lot of necessary neurotransmitters in the body are generated by the gut microbiota, exerting influence on the human body including the brain, among which many of neurotransmitters in the human gut microbiota are also critical molecules.[73]

Intestinal mucosal barrier and blood-brain barrier (barrier system)

Evidence from rodent studies showed that stress changed intestinal mucosal barrier function, made LPS and other cytokines entering blood circulation, and stimulated TLR4 and other TLRs producing inflammatory cytokine.[74] Peripheral produced inflammatory factors could increase the permeability of blood-brain barrier, thus make it possible for peripheral produced inflammatory factors to directly influence the brain.[75]

Therefore, vast evidence of animals and human studies showed that gut microbiota plays a critical role in the brain development and function.[76,77]

Use of Microbiome and Metagenomics to Analyze Composition and Characteristics in Gut Microbiota Respectively

Evidence of the animal studies showed that gut microbiota composition and metabolic products could be obtained through feces analysis.[73]

Microbiome define the composition and functional characteristics of gut microbiota

To effectively understand the role of symbiotic microorganisms of mammals, in particular bacteria, on health and disease, terms and indicators must be used to describe complex ecological gut microbiota. Some bacteria phyla represent its characteristics in gut, and symbiotic bacteria represent the possible diversity, there are about 1000 different bacteria in the gut.[78-80] Two main bacteria phyla are *Bacteroidetes* and *Firmicutes*, which account for at least 70–75% of the microbiome.[79-81] There are also *Proteobacteria*, *Actinomyces*, *Fusobacterium*, and *Verrucomicrobia*, which are relatively small in number.[81] How to describe a variety of gut microbiota? Scientists use microbiome to give an overview description, and microbiome refers to the living bacteria in the gut and its genetic materials, including the archaea, protozoa, fungi, and virus.[41,82]

Dynamic characteristics and diversity of microbiome are beyond people's imagination. Currently, scientists have only begun to understand the distribution and diversity of bacteria phyla which are helpful for health and disease. Metagenomics approach has already revealed that certain bacterial colonies could be as phenotype which shared with a human.[81] Beyond hierarchical features of bacteria phyla, detailed analysis showed that there was a big bacteria variation between the individuals who were relating or nonrelating.[82,83] The microbiome is a dynamic entity, influenced by factors such as gene, diet, metabolites, age, geography, use of antibiotics, and stress.[26,84-91] Hence, the characteristics of gut microbiota are a good representative of individual's environment, which is helpful for understanding individual disease risk, disease progression, and treatment effect. These tools are now being used in the human and animals studies.

Metagenomics is used to be an appropriate technique to characterize gut microbiota

The recent developments in molecular biology and metagenomics allow researchers to better understand the

structure and function of gut microbiota. Metagenomics is an emerging subject that uses the method of nonmicrobial culture to study microbial colony in the environment,[92] the main research objects are bacteria, archaea, fungi, viruses, and other microbes in bacterial colony, and its main purpose is to reveal the deeper genetic and evolutionary laws through analysis of aspects of microbial diversity, population structure and its dynamic change, relationship between members, and the relationship with environment within microbial colony. Metagenomics allows us to discover new genes and proteins, or even new method that is more accurate than traditional microorganisms or molecular biology, and complete comprehensive nonculturable microbial genome in a shorter time.[92]

The differences between metagenomics sequencing and 16S/18S rDNA sequencing are as follows:[93] 16S/18S rDNA gene sequencing is mainly bacterial 16S rRNA or fungal 18S rRNA gene sequencing, and the cores of study are species taxonomy, species abundance, and system evolution within samples. Metagenomics sequencing takes microbiota genome in the environmental samples as research object, directly extracts DNA of all microbiota from environmental samples, constructs metagenomic library, uses high-throughput sequencing technology to analyze population genetic composition, function and participated metabolic pathway of all microbiota contained in environmental samples, interprets the diversity and abundance of the microbial population, seeks for the relationship between microbiota and the environment and relationship between microbiota and the host, and explores and studies new genes with specific functions.

The metagenomic study can be used for evolution analysis, gene discovery, environmental and ecological research, and disease and individual medicine,[78,94,95] especially in individual medical field. Vast evidence showed that population and diversity of human gut microbiota have obvious correlation with the occurrence of human diseases, such as obesity, cardiovascular disease, and tumor, but the impact on health and disease of the human brain is underway.

Conclusion

In summary, gut microbiota-brain axis is a "bottom-up" term as opposed to a "top-down" term of "brain-gut-microbiota axis", no matter what is called, its meaning refers to a bidirectional communication network between gut and brain. Its composition includes gut microbiota and their metabolic products, ENS, sympathetic and parasympathetic branches, neural-immune system, neuroendocrine system, and central nervous system. Moreover, there might have possible five routes of communicating between gut microbiota and brain, including the gut-brain's neural network, neuroendocrine-HPA axis, gut immune system, some neurotransmitters and neural regulators synthesized by gut bacteria, and barriers including intestinal mucosal barrier and blood-brain barrier [Figure 1]. In this communicating network, the brain affects gut movement, sensory and secretion function, and viscera signal from the gut also affects brain function. For example, incoming and outgoing branches of VN play an important role in gut message transmission. Vagal activation has anti-inflammatory effect. Positive effects of many gut microbiota and probiotics on brain function are dependent on the vagal activity.

It is believed that the gut microbiota-brain axis will provide more information and possible route for people to know the brain, understand the brain, and protect the brain.

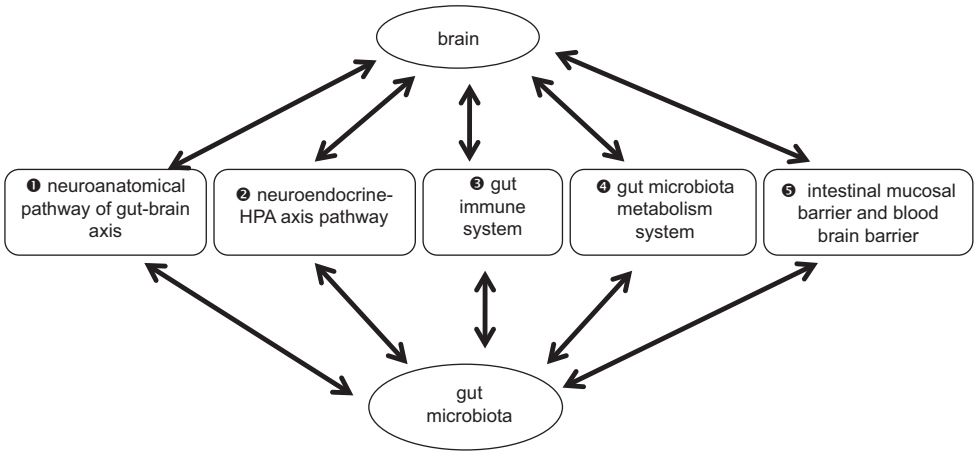

Figure 1: Gut microbiota-brain axis. Five possible communication routes (❶–❺) between gut microbiota and brain: intestinal mucosal barrier and blood-brain barrier (❺) is the important base for neuroendocrine-HPA axis pathway (❷), gut immune system (❸), and gut microbiota metabolism system (❹). Substances produced by neuroendocrine-HPA axis pathway (❷), gut immune system (❸), and gut microbiota metabolism system (❹), only into the system circulation and brain through the intestinal mucosal barrier and blood-brain barrier system can play effect of gut microbiota on the brain. HPA: Hypothalamic-pituitary-adrenal.

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Conflicts of interest

There are no conflicts of interest.

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