REVIEW

THE GUT-BRAIN AXIS: THE CORRELATION BETWEEN STRESS AND GUT MICROBIOME

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Abstract. *Although it was thought that the gut microbiome affects gut physiology only locally, it becomes clearer that these trillions of organisms that reside in the gastrointestinal tract of a human being have a more complex function. Preclinical studies have shown that the microbiome has the ability to interact with the brain in various ways. There have been at least three different channels of communication that favour bidirectional interaction between the brain and the gut. The aim of this review is to summarize the connection between the gut microbiome and the brain, highlighting the process in which stress, in its various forms, can affect the homeostasis of the gastrointestinal tract. Modifications in the gut-brain-microbiome interactions have been analysed and determined in several rodent models of digestive and neurological disorders. The manner in which this information can apply to human beings, is yet to be discovered. Taking all things into account, it is clear that a better understanding of this means of communication could open the door for future therapies for gastrointestinal conditions.*

Keywords: *gut microbiome, gut-brain interactions, microbial signalling, stress-induced alterations.*

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Abbreviations: GB= Gut-Brain; GI= Gastro-intestinal; IBS= Irritable bowel syndrome; ENS= Enteric nervous system; CNS= Central nervous system; ANS= Autonomic nervous system; SCFAs= Short-chain fatty acids; 2Bas= Secondary bile acids; TLRs= Toll-like receptors; MAMPs= Microbe-Associated Molecular Patterns; HPA= Hypothalamic pituitary-adrenal; SPF= Specific pathogen-free; GF= Germ free; BMI= Body-mass index; CFU= Colony forming unit; MODS= Multiple organ dysfunction syndrome; SIRS= Systemic inflammatory response syndrome; L/D test= Light-Dark box test.

1. Introduction

Discussions revolving around the importance of the human microbiome have recently gained great popularity among scientists. Although questions regarding the subject continue to emerge, there is yet not sufficient information to define the exact mechanisms and pathophysiology of how the gut microbiome influence general health. As in present days, the mental health of the human population has become extremely valued, as a great number of people struggle with problems in day-to-day life. For that reason, it is imperative to learn more about the correlation between stress and the gut microbiome, as well as the latent issues that

can arise from the imbalance of the microbial species in the human intestines.

2. The importance of gut microbiome

The human gastrointestinal system is home to trillions of bacteria, viruses, fungi, archaea, and eukaryotic species, which are together referred to as the gut microbiome. These organisms, which may weigh up to 2 kg in an ordinary adult, account for more than 50% of the cells in the human body [1]. Due to the acidic environment, the presence of bile and pancreatic juice, and the effects of peristalsis that prevent sustained colonization, the stomach and small intestine contain few organisms. Therefore, the vast majority of the microbiota reside in and communicate with the human host in the colon. In the past ten years, due to increasing availability and lower costs, our understanding of the human gut microbiome in both health and illness has dramatically risen [2].

Even though one cannot entirely define a healthy microbiome, there are a few standards that one may consider. Relevant characteristics are the diversity of the microbial species, stability, resistance to stress-related change (antibiotics, infection, immunosuppression), and a high level of redundancy in metabolic pathways [3].

3. The gut-brain axis

Food intake, immune function, and sleep are all regulated by bidirectional interactions between the gut and the brain (GB). Even though modifications of GB interactions have for quite some time been hypothesized to be the cause of chronic abdominal pain symptoms and gastrointestinal dysfunction, the correct terminology (disorders of the GB interaction instead of functional GI disorders) has only recently been acknowledged by experts [4]. Even though research over the past ten years has accomplished significant information about the pathophysiology of GB disorders like irritable bowel syndrome (IBS) and functional dyspepsia, there is still disagreement about the relative contribution of peripheral (such as the gut) and central (such as the brain, spinal cord) mechanisms to the generation of symptoms in these diseases and other comorbid syndromes like functional chest pain and functional abdominal pain. Nonetheless, there is developing agreement that the pathology of persistent stomach pain can be understood as a dysregulation of the interaction between signalling in the stomach, intestinal microbiota, enteric nervous system (ENS), and central nervous system (CNS). This process is believed to occur at the same time with modifications in gut motility and regional transit, visceral sensitivity, immune function, and mood [5].

The newly discovered interaction between the gut and the brain is thought to be responsible for different brain disorders whose pathophysiology had been formerly correlated to mechanisms limited to the brain. Preclinical and clinical studies suggest that treatment of gut dysbiosis could become a reasonable solution for defective GB interactions, as well as for psychiatric and neurological disorders (depression, anxiety, Alzheimer's disease, Parkinson's disease, and autism spectrum disorders [5].

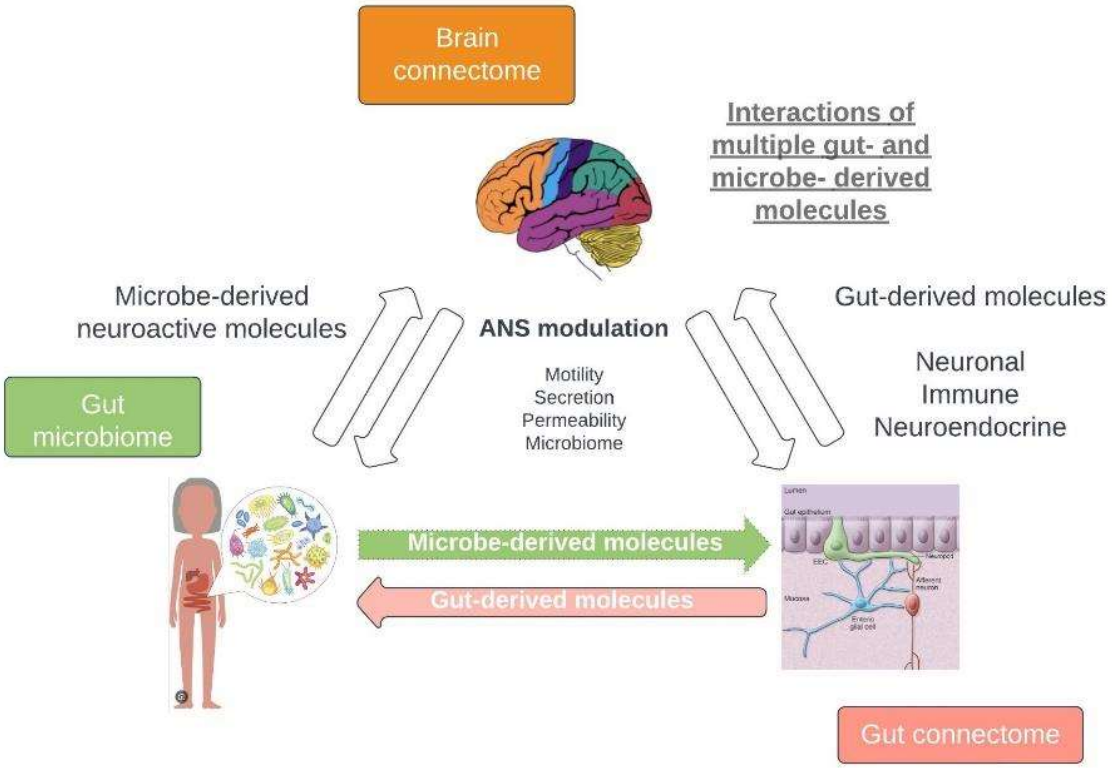

Figure 1. Interaction of multiple gut-and microbe- derived molecules [5] – The autonomic nervous system modulates motility, secretion, permeability and the microbiome as a result of signalling between Microbe-derived neuroactive molecules and Gut-derived molecules. *(ANS: Autonomic nervous system)*

Gut microbiota and brain signaling: interoception

Until recently, signals from the gut to the brain were considered to be transmitted only via fine unmyelinated vagal and sympathetic afferent fibres. Research in the microbiome sciences has concluded that interoceptive information could be transmitted to the brain via different microbes and microbial derived mediators [6].

Microbial-derived intermediates, such as short-chain fatty acids (SCFAs), secondary bile acids (2BAs), and tryptophan metabolites, interact with the central nervous system through different pathways. Some of the metabolites activate directly the enteroendocrine cells, enterochromaffin cells, and mucosal immune system to produce bottom-up signals, while other metabolites can infiltrate through the intestinal barrier and pass into the systemic circulation and could even overpass the blood-brain barrier. It is yet not entirely understood whether the concentration of these intermediates could reach a certain value that is sufficient for the activation of certain brain circuits. [6]

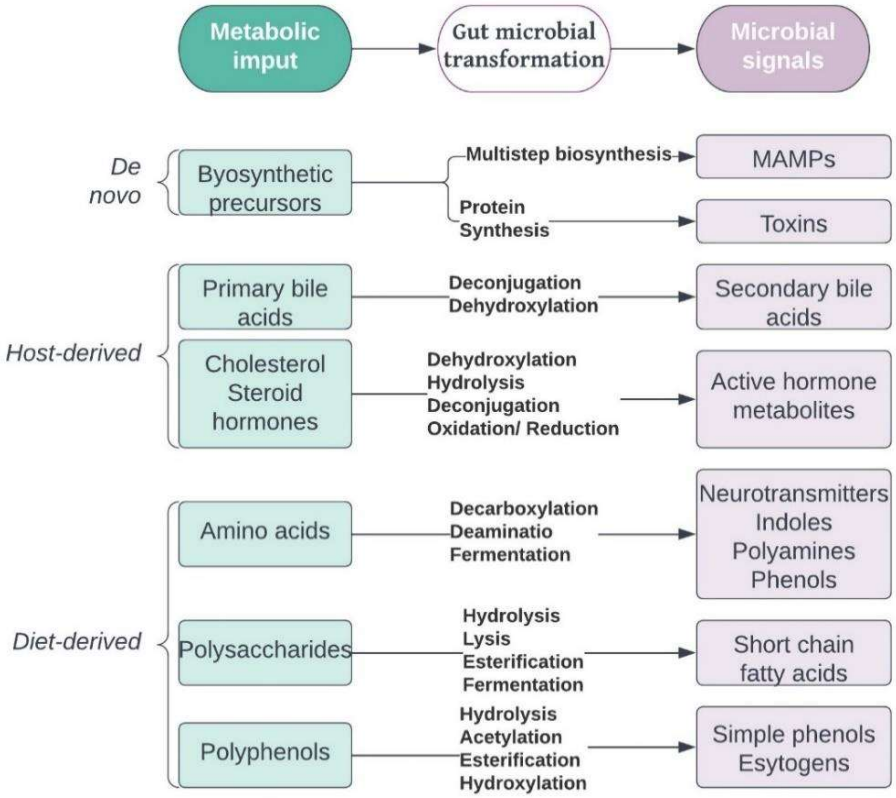

Figure 2. Means of microbial signalling [7] – Gut microbiota promotes the transformation of different molecules (De novo, Host-derived, Diet-derived) into microbial signals. Biochemical mechanisms are listed in the third column. *(MAMPs: Microbe-Associated Molecular Patterns)*

Figure 2 resumes the three main pathways through which the brain can receive information from the gut-derived molecules.

In immune signaling, parts of the microbial membranes (lipopolysaccharides, MAMPs) or even intact microorganisms, can either activate the TLRs on microglia or neurons by reaching the brain through systemic circulation or stimulate the TLRs on immune cells that are present in the gut. As a result, cytokine secretion is triggered locally as well as systemically. Host-derived molecules such as primary bile acids, cholesterol, and steroid hormones are excreted into the lumen of the small intestine and converted through different chemical interactions into neuroactive metabolites that can be reabsorbed in the circulatory system. Molecules resulting from diet, such as amino acids, polysaccharides, and polyphenols, are also modified into different molecules that can act as neurotransmitters [7].

4. Correlation between stress and gut microbiome

I. Stress

The definition of stress could be stated as the human body's reaction to the difficulties that one encounters in a certain environment. The agents that produce stress could vary in different ways. Stressors could be acute, chronic, acute on chronic, as well as repetitive acute. Depending on each person, the response to stress could differ substantially from one person to another. All things considered, stress plays a part in the susceptibility to numerous illnesses and disabilities. Thus, it is important to examine the impact of stress on human general health, as it also raises concerns for global economy.

A. Alterations in hypothalamic-pituitary-adrenal (HPA) function

Psychological and physical factors that contribute as stressors activate through different mechanisms, including hormonal changes. Firstly, they trigger the release of corticotropin-releasing hormone by activating the hypothalamic-pituitary-adrenal (HPA) axis. As a result, the adrenocorticotrophic hormone is released systemically, which then stimulates the glucocorticoid synthesis (cortisol) in the adrenal cortex [8]. Moreover, stressors induce the secretion of catecholamines (noradrenaline and adrenaline). Finally, the gastro-intestinal tract as well as the gut microbiota have the ability to react to stress and stress mediators. Bacteria in the gut are sensitive to different stress-related neurochemical mediators. By this means, the body is susceptible to bacterial infection [9]. Recent findings theorize that bacteria act as delivery vehicles for neuroactive compounds, and therefore can affect host physiology by producing neurochemicals [10].

Reversely, the influence of the gut microbiome on the HPA axis has been studied by different researchers who analyzed rodent models. Studies conducted on mice in 2014 revealed a direct connection between the microbiota and the HPA axis. There is evidence that there is an exaggerated corticosterone and adrenocorticotrophin response to stress in germ-free (GF) compared to conventionally house-specific pathogen-free (SPF) mice. GF have no commensal microbiota, which leads to a not sufficiently developed immune system [11]. In the past years, more and more information has been gathered that suggests the significant impact of the microbiota on modulating the HPA axis early in life. At birth, the stress response system is functionally immature, and it continues to develop in the postnatal period, at the same time as the colonization of the intestinal tract. In an experiment conducted on maternally separated mice, treatment with probiotics (Lactobacillus sp.) showed a significant role in reducing corticosterone levels, which had been elevated by psychological stress [12].

B. Direct influences on stress circuits

Neuronal activation of stress circuits by the microbiota has been proven to occur not only by modulating the HPA axis but also in a direct manner. Research proves that in the acute phase of the infection with *Campylobacter jejuni* in mice, induction of the neuronal activation marker cFOS was clear in the vagal sensory neurons, even when a systemic immune response was not present [13].

II. Physiological stress-exercise

High-intensity exercise has been proven to be another stressor that could determine gastro-intestinal imbalances. 30 to 90% of long-distance runners admitted in some reports that they experienced intestinal distress in relation to exercise [14]. Symptoms can vary from mild to severe and consist of nausea, vomiting, abdominal angina, and bloody diarrhea. Intensive exercise has been correlated with reduced gastro-intestinal blood flow, hyperthermia, and hypoxia, contributing to different modifications in the gut microbiome. Regarding the athletes, besides intensive workouts, another factor plays a significant role in the alterations of the gut microbiome: dietary patterns. Clarke et al. proved the effect of exercise and dietary plans on the gut microbiota [15]. Improved microbial diversity was demonstrated and positively correlated to increased protein intake and increased exercise. In comparison to two non-athletic control groups (size matched, high BMI – 30 kg/m^2 and age/gender matched), Clarke discovered lower levels of inflammatory markers and improved metabolic markers in athletes. The diversity of the microbiota was positively correlated to high protein intake and high plasma creatine kinase levels, which suggests that diet as well as high-intensity exercise influence changes in microbial diversity.

Microbial diversity was reflected by the presence of representatives of 22 phyla of bacteria, in contrast to 11 to 9 phyla in the two control groups [15].

Physical activity and fitness have the potential to affect the microbiota through various mechanisms. Intense exercise generates numerous metabolites and inflammatory mediators, whereas regular exercise and fitness can suppress basal inflammatory cytokines, indicating a feedback loop between exercise biology and host immunity [16]. Consistent physical activity has an anti-inflammatory impact that enhances the immunological profile in conditions like type 2 diabetes mellitus, coronary artery disease, peripheral arterial disease, and obesity. In animal models, repeated exercise lowers the expression of pro-inflammatory cytokines, while increasing the expression of anti-inflammatory IL-10 [8]. Furthermore, regular exercise can reduce oxidative damage to the colon in a rat model of colitis [17].

Contrasting the beneficial effect of habitual exercise, extensive exercise can negatively influence the gastrointestinal tract and its function. Due to hypoperfusion, which is present during high-intensity exercise, intestinal ischemia could result. Endotoxin translocation could also emerge as intestinal permeability increases. Recently, scientists have considered analyzing the effect of probiotic supplements on preventing gastro-intestinal symptoms such as nausea, cramping, bloating and diarrhea. Products containing Lactobacillus and/or Bifidobacterium species have been prescribed to athletes for one to six months before and/or after exercise or a competition (at varying doses of 109–1012 CFU/day). Some studies demonstrated clinical outcomes of improved upper respiratory tract illness and gastrointestinal illness, as well as immunological measures and outcomes [8].

III. Physiological Stress-Critical Illness

Similar to high intensity exercise, the intestinal hypoperfusion resulting from the redistribution of splanchnic circulation could be severe enough to cause ischemia and mucosal injury. This process could occur, in particularly in critically ill individuals. In those cases, the gut as well as the microbiome play a role in developing severe infectious complications or multiple organ dysfunction syndrome (MODS). A recent study conducted by Shimzu *et al*. analyzed the fecal pH and the presence of organic acids in patients with systemic inflammatory response syndrome (SIRS) [18]. Results suggested that these patients had reduced total anaerobic bacterial counts (especially 2–4 log fewer commensal *Bifidobacterium* and *Lactobacill us*), as well as 2 log higher potentially pathogenic *Staphylococcus* and *Pseudomonas* group counts. Moreover, results showed lower concentrations of organic acids (especially Short-chain fatty acids (SCFA) acetate, propionate, and butyrate) as well as a high fecal pH in the group of critically ill patients. Alkaline and acidic pH have been associated with lower concentrations of Bacteroide and Bifidobacterium species, as well as a higher incidence of bacteremia, in comparison to the study group with normal pH. A pH greater than 6.6 has been correlated with a greater incidence of bacteremia and mortality rates. Total SCFA levels decreased with pH>6.6.

It remains unclear whether the observed changes are a result or a cause of SIRS. Although this study suggests that fecal pH could serve as a risk factor indicator, it has some limitations, such as its use of culture-based microbiota analysis and lack of specification of gastrointestinal region-specific pH levels. Research has demonstrated that the gut microbiome undergoes modifications within 6 hours of a metabolic insult and that it fails to revert to the microbial patterns observed in healthy controls [19].

Numerous meta-analyses and systematic reviews have been conducted to investigate the use of probiotics in critically ill patients. However, the outcomes of such studies may vary depending on which studies are included in the analysis. Nonetheless, it appears that the administration of probiotics to critically ill patients is associated with positive outcomes [8].

IV. Psychological Stress

Researchers developed the gut-brain axis concept at the same time that one noticed that the digestive process could be regulated by the endocrine system through different hormones. Functional Gastro-intestinal diseases such as irritable bowel syndrome and functional dyspepsia have been explained by the reciprocal action of psychological factors and altered gut physiology, the gut-brain axis. Stress that happens early in life (e.g., psychological, sexual and/or physical abuse) has been said to considerably impact the gastro-intestinal normal functions, as the period of time when the gut microbiota is developing the most is at the beginning of life.

Different studies conducted on animals suggested that psychological stress significantly influences the gut microbiome and leads to a dysfunctional gut-brain axis. Maternal separation, a model for early life stressors, has been linked to extended HPA-axis hyperactivity, anxiety-like behavior, visceral hypersensitivity, and dysfunctional cholinergic activity in the gut, as well as high permeability of the gut barrier. By attempting to restore the dysbiosis in the gut, researchers advanced probiotic treatment, that may influence the development of common functional gut disorders. In addition to this process, stress in early life has been demonstrated to promote morphological changes, such as increased number of goblet cells in the crypts of the proximal colon and subsequent amounts of mucus production and a thinner mucosal layer. Alterations in gut microbiota composition in animals that have been separated from their mothers may be caused by modifications to both gut physiology and morphology.

The reverse action of this interaction is that dysbiotic gut microbiomes cause anxiety and depression. Metabolites synthesized in the gut appear to influence brain biochemistry and behavior [8].

Clinical evidence has been published in a limited number of studies that made use of probiotics to analyze their effects on stress-related disorders. Diop et al. explored the effect of a probiotic preparation treatment of 3 weeks (Lactobacillus acidophilus and B. longum) on volunteers that experienced stress-related symptoms (anxiety, nervousness, irritability, sleeping problems, and GI disturbances) during the previous month. The probiotic seemed to provide a benefit to these individuals affected by chronic stress. A double-blind, placebo-controlled study conducted in 2016 by Kato-Kataoka showed that oral administration of L. casei strain for 8 weeks, to medical students before an academic examination contributes to the prevention of cortisol hypersecretion and stress-related symptoms. Moreover, in different research (Yang *et. al.* 2016), probiotic administration (Clostridium butyricum) to patients who were scheduled for an elective surgery (laryngectomy) ameliorated the clinical anxiety and negative effects of stress compared to placebo [20].

V. Gut-brain axis and behaviour

Different studies conducted on altered commensal intestinal microbiota, either germ-free mice, or conventionally housed animals treated with either probiotics and/or antibiotics or infected with pathogenic bacteria, show that behavioral changes occur when the gut microbiome is modified. Different strains of mice have been analyzed, as genetic background has a significant impact on the behavior of the rodents [11]. For example, there is evidence that administering low levels of pathogenic bacteria orally increased anxiety-like behaviour in the CF-1 strain, as assessed by the holeboard test [21].

Moreover, infection with the parasite Trichuris muris increases anxiety-like behavior on Balb/C and AKR strains (L/D test) [22]. On Sprague-Dawley male rodents, probiotic treatment reversed the impact of maternal separation on depressive-like behavior in rats in FST [23].

The gut microbiota can influence behavior by modulating neurotransmitter metabolism and other pathways that are yet to be fully defined via regulation of the vagus nerve. Consequently, probiotics and symbiotics may be a promising therapeutic option, either as a stand-alone or adjunct therapy to conventional treatment, for patients with irritable bowel syndrome who also have depression or anxiety [8].

Even though, treatment with probiotics in animal research has consistently proven to have a role in modulating anxiety- and depressive-like behavior, there is not enough evidence regarding their effects on psychiatric concerns in humans. Thus, in the limited number of preclinical studies, it becomes clearer that the use of probiotics might have similar effects on humans, as it has on animals [11].

5. Conclusions

To sum up, according to research, the stress effect on the gut microbiome and the gut microbiome's modulation of stress have become clearer in the past few years. As research consists of an abundance of preclinical studies, there are still not sufficient analyses on human beings. The challenge is, therefore, to translate the evidence from preclinical studies to healthy human participants in various stressful settings. Probiotics seem to be a promising intervention for stress-related disorders.

Author Contributions:

I.M.V. conceived the original draft preparation. I.M.V. was responsible for conception and design of the review. I.M.V. was responsible for the data acquisition and for the collection and assembly of the articles/published data, and their inclusion and interpretation in this review.

Compliance with Ethics Requirements:

"The author declares no conflict of interest regarding this article".

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