Minireview

The microbiota-gut-brain axis: An emerging role for the epigenome

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Impact statement

Alterations in the composition of gut microbiota may influence the etiology of gastrointestinal and neurological disorders by disturbing the communication in the gut-brain axis. Epigenetic changes in the gut-brain axis may perpetuate these phenotypes even when the gut microbiota has been restored. The studies reviewed in this article provide an overview of the influence the microbiota exerts onto its host's epigenome. First, we summarize the bidirectional pathways through which the microbiota and the gut-brain axis communicate. Second, we provide evidence for the epigenome-altering capacity of the gut microbiota. Finally, we address the existing knowledge gaps and highlight the potential role of the epigenome in the microbiota-gut-brain axis.

Abstract

Through the gut-brain axis, the microorganisms that reside in the gut are able to exert an important influence on the central nervous system. Preclinical and clinical evidence suggests that alterations in the composition of the gut microbiota are involved in gastrointestinal and neurological disorders. During critical neurodevelopmental time periods, such as the early life, changes in gut microbial composition may detrimentally impact neurodevelopment, and subsequently lead to neurological disorders in later life. The finding that neurological disorders persist suggests that epigenetic modifications may be involved in response to disruption of the microbiota-gut-brain axis. Through establishing epigenetic modifications, environmental (microbial) signals can interfere with the cellular gene expression patterns. These long-lasting modifications exert their effects even when the initial stimulus is removed. In this review, we discuss the pathways that provide bidirectional communication between the microbiota and the central and peripheral nervous systems. Furthermore, we summarize how these microorganisms in the gut exert their influence through changing the epigenome in the brain-gut axis.

Keywords: Gut-brain-axis, microbiota, germ-free animals, epigenetics, central nervous system

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Introduction

Substantial amounts of epidemiological evidence exist for an association between neurological and gastrointestinal (GI) disorders. For instance, patients who suffer from anxiety issues, major depressive disorder (MDD), or autism spectrum disorder (ASD) often also report GI complaints. Vice versa, GI disorders, such as ulcerative colitis, Crohn's disease, and irritable bowel syndrome (IBS), are often co-morbid with anxiety issues and/or depression. These epidemiological findings introduced the concept of the "gut-brain axis," an extensive communication network that links the GI tract with central cognitive and emotional centers within the central nervous system (CNS).[1] In this way, the brain can influence gut epithelial transport, intestinal permeability, GI motility, and visceral sensitivity. Concomitantly, signals arising from the gut are capable of triggering neurodevelopmental and neurobehavioral effects on the brain.[2,3] This bidirectional influence is fundamental in the maintenance of homeostasis in both the GI system and the brain. The numerous microorganisms living in the gut, belonging to different classes of viruses, archaea, protozoa, bacteria, fungi, and eukaryota, are a long-overlooked component of the gut-brain axis.[4] The presence of these microorganisms is essential for gut homeostasis. However, these microorganisms also undeniably influence central and peripheral neural processes,

neurodevelopment, and behavior.[5–8] Patients with GI and/or neurological disorders often show a differently composed gut microbiota when compared to healthy controls. In these patients, often overall microbial diversity is reduced, certain microbial strains are over- or underrepresented, whereas other strains may be completely absent, shifting the balance towards an "unhealthy" microbiota.[9–14] Transfer of the fecal microbiota from patients with IBS or MDD to germ-free (GF) animals also transferred IBS-like visceral pain and depressive-like behavior to these animals.[15,16] These studies showed the importance of the microbiota to induce behavioral alterations, but also indicated that the transient nature of the microbiota can be exploited. Restoring a healthy microbiota may ameliorate neurological maladaptation. In animals and in IBS-patients, supplementing the diet with pre- and probiotics ameliorated visceral hypersensitivity.[17–20] Similar to IBS, certain probiotics, prebiotics and antibiotics are able to ameliorate depression-like behavior in animals and patients.[21–25] In our current understanding of the microbiota-gut-brain axis, it is thought that detrimental changes in the composition of the gut microbiota that occur during critical developmental time windows, can negatively impact neurological and/or behavioral development.[26] Interestingly, although these microbiota composition changes are often transient, they can induce long-lasting changes in the gut-brain axis. An underlying mechanism through which transient changes can be embedded for the long-term, is through modulating the epigenome of the nervous system. Recent studies are uncovering that epigenetic changes, regulating long-term changes in gene expression, in the gut-brain axis perpetuate transient changes in the gut microbial composition (Figure 1).

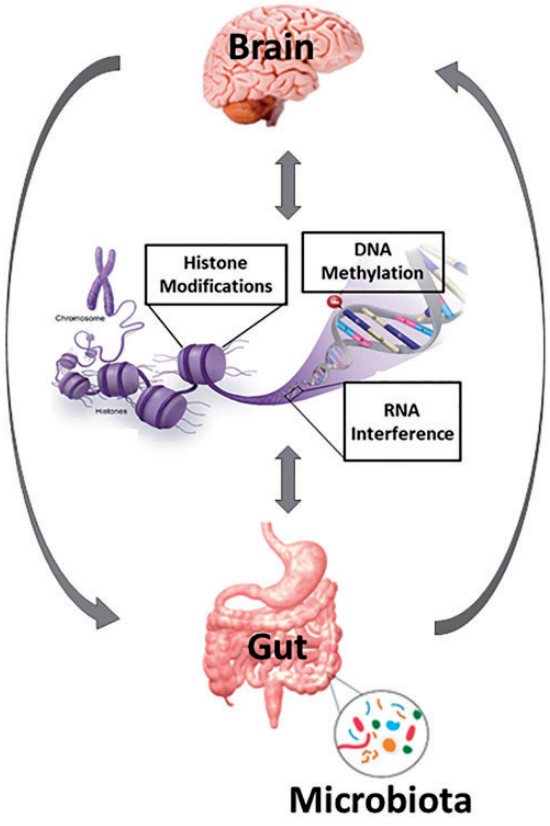

Figure 1. Microbiota–gut–brain axis. The bidirectional communication between the gut and the brain exists of multiple pathways. Epigenetic changes, under influence of the gut microbiota, occur both in the gut as well as in the brain.

Communication in the microbiota-gut-brain axis

The communication in the microbiota-gut-brain axis is bidirectional and consists of many direct and indirect pathways. Interestingly, through its presence in the gut, the microbiota influences all of these routes of communication. As a result, the microbiota can influence CNS activity and certain CNS activity can alter microbial composition and activity. Direct communication in the microbiota-gut-brain axis occurs through the nervous system. The gut is innervated by the enteric nervous system (ENS), the sympathetic and parasympathetic branches of the autonomous nervous system (ANS), and sensory nerves of the CNS.[27] Information from the ENS is transmitted via the vagus nerve via the nucleus of the tractus solitarius to the dorsal motor nucleus of the vagus in the medulla of the brainstem. Visceral sensory nerves in the gut transmit afferent signals via the spinal cord to the CNS. In the brain, afferent signals are relayed to different sites, where processing occurs and efferent signals back to the gut generated.[28] The microbiota interacts directly with these afferent neurons through the release of neurotransmitters in the gut that can stimulate intrinsic primary afferent neurons in the gut, or directly stimulate the extrinsic primary afferent neurons in the vagal, pelvic, and spinal afferent nerves: acetylcholine and gamma aminobutyric acid (GABA) are produced by *Bifidobacterium* and *Lactobacillus* species, while serotonin, dopamine, and norepinephrine (NE) are produced by *Escherichia*, *Streptococcus*, and *Enterococcus* species.[7,29] Vice versa, microbial populations, that express receptors for GABA, NE, and serotonin, are able to pick up neurotransmitters released from motor or efferent signals coming from the brain. In this way, CNS activity can directly regulate microbial activity in the gut.[30–33]

Through local interactions with neurons, the microbiota modulates gut homeostasis. In addition, the microbiota is an important source for certain neurotransmitters and neurotransmitter activity, and its influence extends far beyond the gut environment. For instance, the biosynthesis of serotonin in the enterochromaffin cells is controlled by indigenous spore-forming bacteria. In absence of these bacteria, the release of serotonin in the GI tract is significantly lowered.[34] Furthermore, serotonin concentrations in the serum and the certain brain regions, such as the hippocampus, are decreased.[35] Moreover, the presence of the gut microbiota is essential for normal neurotransmitter turnover rate in the CNS. In the hippocampus, frontal cortex and striatum of GF animals, the turnover rates of noradrenaline, dopamine, and serotonin are increased.[30,36,37] Hence, neurotransmitter bioavailability, neurotransmitter turnover rate, and normal microbial-neuronal interactions are essential for the communication between the CNS and the

gut microbiota. Disruption of the microbiota composition may affect all of these pathways: alterations in microbiota-neuron interaction and signal transmission in the CNS, may eventually impact CNS functionality.[38,39]

Indirect communication in the microbiota-gut-brain axis involves neuroendocrine systems, the immune system, and secretion of metabolites. Alterations in these pathways often change the gut environment or directly influence microbial composition. The major neuroendocrine system involved in stress-induced GI physiology, is the hypothalamic-pituitary-adrenal (HPA) axis. Under stressful conditions, activation of the HPA axis increases levels of circulating cortisol (CORT) in the body.[40] The stress hormone CORT directly and indirectly influences gut physiology by changing the activity of regulatory regions in the brain, immune cells, smooth muscle, and gut epithelial cells.[41–43] In adult rodents, psychological or social stress activated the HPA axis, which altered gut physiology and microbiota composition.[44–46] Interestingly, the gut microbiota is necessary for the correct formation of the HPA axis. GF mice show higher levels of HPA axis activity (higher basal CORT production or exaggerated CORT production under stress) when compared with controls. In GF mice, gut colonization with *Bifidobacterium infantis* normalized the exaggerated HPA response to restraint stress, whereas colonization with the *Escherichia coli* further exacerbated the HPA response to restraint stress.[47] Additional evidence in rhesus monkeys showed that prenatal exposure to CORT changed the microbiota profile of the newborns.[48] Postnatal premature activation of the HPA axis due to maternal separation stress, predominantly reduced the number of Lactobacilli, a strain of bacteria that is important for normal functioning of colonic mucosa. The reduction in these beneficial microorganisms could have compromised colonic mucosal function, and made them more susceptible to opportunistic bacterial infections.[49]

Although our gut microbiota consists of many beneficial microorganisms, it also harbors detrimental organisms. Hence, our immune system needs to be primed to only respond to the presence of detrimental microorganisms. Several bacterial strains produce short-chain fatty acids (SCFAs) which can be taken up by circulating monocytes. In these immune cells, SCFA favors the production of anti-inflammatory cytokines and prostaglandin E2.[42] Moreover, SCFA induces a shift towards an anti-inflammatory phenotype: SCFA administered to a murine macrophage cell line (RAW264.7) not only prevented the production of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6) after lipopolysaccharide (LPS) exposure, but also increased anti-inflammatory cytokine (IL-10) production.[50] This shift to an anti-inflammatory state in the gut is also known to modulate neuronal development.[51]

Substantial evidence indicates that microbial SCFAs also regulate enteric glial and neuronal functionality.[52] For example, enteric neurons express the type 2 monocarboxylate transporter (MCT2) or other G protein-coupled receptor that can interact with SCFA butyrate. As a result, butyrate activates pathways that ultimately elevate choline acetyltransferase concentrations in enteric neurons. Other SCFAs interact with the sympathetic nervous system through binding the G protein-coupled receptor 41 (GPR41) on these neurons. This interaction generates action potentials and results in the release of NE from the sympathetic nerve terminals.[53–55]

In summary, through direct or indirect interaction with neurons, the microbiota can modulate or alter the colonic signals the brain receives. Moreover, the microbiota can influence how the CNS processes this and other incoming information and the generation of top-down signals. Signals originating from the brain can alter gut motility and permeability, which can change the gut environment and, in this way, influence the composition of the microbiota.

Emerging role of epigenetic reprogramming in the microbiota-gut-brain axis

Histone tail modifications, DNA methylation, and non-coding RNAs are the most known and best studied epigenetic mechanisms. Through epigenetic modifications, developmental or environmental signals are integrated into the cell's gene expression profile, without interfering with the DNA sequence itself. Often, these modifications do not need the external signals to remain in place, and lead to stable long-term changes in gene expression patterns. As the microbiota is part of the environment, it can interact with the host's genome by modifying the host's epigenome in the gut-brain axis. In this way, the absence or changes in microbiota composition can establish long-lasting epigenetic modifications that can ultimately affect behavior. Although epigenetic modifications are usually long-lasting, they do not need to be permanent. Therefore, as epigenetic modifications are reversible, they can be changed at later stages. As a consequence, epigenetic modifications serve to adapt or fine-tune the cellular gene expression profile to environmental signals. Interestingly, restoration of the gut microbiota during critical neurodevelopmental periods or supplementation with pre- or probiotics has beneficial influences on gene expression and behavior. These beneficial changes might have been caused through modulation of the host's epigenome.

The epigenome is influenced by the gut microbiota

Several metabolites, produced by the gut microbiota, can directly or indirectly interact with the host's epigenome. Bacteria from the genera *Eubacterium*, *Clostridium*, and *Butyrivibrio* are a major source for the SCFA butyrate, which can act as a histone deacetylase (HDAC)-inhibitor. Butyrate receptors are expressed by multiple cell types throughout the body.[56] For instance, the colon, liver, and white adipose tissue of GF mice showed different histone 3 and histone 4 tail acetylation and methylation patterns, when compared to control animals. The epigenetic changes were reversed after colonization with a normal microbiota, or after supplementation of the diet of the GF mice with butyrate and other SCFAs.[57] Evidence also suggests that butyrate can cross the blood–brain barrier and change the epigenome in the CNS. In a rat model of depression,

chronic butyrate treatment inhibited histone deacetylases in the brain, which lead to increases in BDNF and amelioration of the phenotype.[58] The presence of certain bacterial species in the gut, such as *Helicobacter pylori* can change the expression levels of epigenetic enzymes. The presence of *Helicobacter pylori* in the gut increases the CpG-methylation in the promoter region of O6-methylguanine DNA methyltransferase, which ultimately decreases the expression of this DNA methyltransferase in the cells of the gastric mucosa.[59] Other bacteria are even capable of secreting proteins with epigenetic properties. Rv3423.1, derived from *Mycobacterium tuberculosis*, functions as a histone acetyltransferase.[60] Moreover, Mycobacterial Rv1988 is capable of methylating histone tails.[61] Apart from directly interfering with the host's epigenome, the gut microbiota also exerts an indirect effect as an important source for essential substrates and cofactors for epigenetic enzymes. Acetyl-CoA and S-adenosyl-methionine, produced by gut bacteria, are indispensable substrates for histone acetylation and DNA methylation. Furthermore, the gut microbiota regulates the absorption and secretion of essential enzymatic cofactors such as zinc, iodine, cobalt, selenium in the gut. In this way, the microbiota further exert its control over the epigenome.[62]

The microbiota influence gene expression

Recent studies have established that the presence of the gut microbiota is essential for normal gene expression in the CNS. Especially regions involved in the development of mood and neurological disorders, such as the amygdala and hippocampus, are dependent on the presence of gut microbes for their normal gene expression. Often, genes involved in neurodevelopment, mood and anxiety disorders, fear learning and extinction, and stress responses are affected by changes in the microbiota. The hippocampus of GF mice showed a disturbed transcription profile of synaptic plasticity genes, serotonin, BDNF, NMDA, the NMDA receptor NR2B, and the glucocorticoid receptor.[22,37] Interestingly, some of these changes occurred specifically in the dentate gyrus of the hippocampus of GF female mice.[63] In the amygdala of GF mice, the expression of genes related to neuronal activity, synaptic plasticity and (cholinergic) transmission, and immediate early genes was notably increased.[64,65] Experimental evidence found that supplementing the diet of mice with *Lactobacillus rhamnosus* changed the transcription of the $GABA_{B1b}$ subunit: expression was increased in cortical brain regions, but decreased in the amygdala, hippocampus, and locus coeruleus. In the same mice, the hippocampus revealed increases in $GABA_{A\alpha 2}$ subunit expression, whereas in the amygdala and prefrontal cortex, expression was reduced.[39]

The microbiota influences gene expression through epigenetic reprogramming

The epigenome in the microbiota-gut-brain axis has not been widely studied. However, recent advancements in this field are starting to unravel the involvement of epigenetics. In the aforementioned studies, the wide-spread dysregulated mRNA expression in the amygdala of GF mice was accompanied by a vast network of dysregulated miRNA expression, which accounted for the observed behavioral changes.[65,66] Specifically, GF mice showed decreases in miR-182-5p and miR-183-5p, which are involved in amygdala-dependent stress and fear-related outputs, and decreases in miR-206-3p, which is known to alter BDNF expression.[66] Colonization on post-natal day 21 partially restored miRNA expression patterns, which only partially normalized impaired amygdala-dependent fear memory recall. miRNA expression was also dysregulated in the hippocampus of GF mice, which led to changes in gene expression related to axon guidance. During the development of the nervous system, axon guidance is necessary to locate and recognize appropriate synaptic partners. Inappropriate wiring of neurons in GF mice may underlie behavioral deficits in GF mice. As with other studies, colonization of the gut of adolescent mice did not reverse behavioral deficits.[67]

Apart from the CNS, other components of the gut-brain axis are also epigenetically affected by the presence of the microbiota. For instance, GF mice fail to develop immune tolerance and lack the intestinal barrier integrity that is commonly associated with the presence of gut microbiota. Especially the absence of *Lactobacilli* and *Bifidobacteria* is important, as these microorganisms are the major source of butyrate. By inhibiting HDACs, butyrate suppresses nuclear NF-κB activation, upregulates PPARγ expression, and decreases IFNγ production in the residing gut immune cells, promoting an anti-inflammatory gut environment.[68,69] This growing evidence suggests that the microbiota can modulate the epigenome in the gut-brain axis. In this bidirectional communication axis, the opposite should also be true: epigenetic changes in the gut-brain axis should also have an impact on the microorganisms residing in the gut. To date, direct proof is lacking, but indirectly the pieces of the puzzle can be put together. For instance, in our laboratory, we showed that chronic psychological stress in rats induces epigenetic changes in the amygdala, an important regulator of the HPA axis, which lead to visceral hypersensitivity.[70] The visceral phenotype could be rescued by infusing HDAC inhibitors directly into the amygdala. Other studies showed that the same stressor affected the microbiota, which was an underlying cause for visceral hypersensitivity.[71] Interestingly, supplementation of the diet with butyrate-producing *Lachnospiraceae* reversed visceral hypersensitivity in stressed rats.[46] Whether this HDAC inhibitor also was able to reach the amygdala to prevent modulation of the epigenome remains to be investigated. The effects of psychological stress extend far beyond the brain, and are also known to influence inflammatory markers through silencing of the NLRP6 gene.[72] The shift towards a pro-inflammatory phenotype in the gut and visceral hypersensitivity was attenuated by administration of *Clostridium butyricum*, which prevented the gene silencing of NLRP6.[73] Animal models of early life stress, such as maternal separation, offer the possibility to interfere with critical (neuro) developmental models. Therefore, the maternal separation model has been widely used to study the disorders of the gut-brain axis

as it leads to increases in gut permeability, gut inflammation, visceral sensitivity, and induces HPA axis hyperreactivity, depressive-like behavior, anxiety, and fear.[74–76] These functional and behavioral changes are mediated by changes in DNA methylation and histone modifications at key genes in the CNS.[77–82] These epigenetic changes are key in the long-lasting behavioral changes and are interesting targets for reversal of the observed phenotypes. Visceral hypersensitivity was ameliorated after intrathecal injections of HDAC inhibitors, whereas dietary methyl donor supplementation rescued depressive-like behavior.[75,83] Concomitantly, maternal separation also changes the composition of the microbiota of separated pups.[84,85] Interventions with pre- or probiotics, during neonatal exposure to maternal separation, were able to attenuate the effects of maternal separation on neurotransmitters in the hypothalamus, intestinal cytokines, anxiety behavior, prefrontal cortex-mediated fear regulation, or visceral hypersensitivity. Prebiotic interventions after weaning also showed to be effective in attenuating visceral hypersensitivity. Interventions that aimed to restore gut barrier function also normalized microbiota composition.[86–90]

Summary and conclusions

The microbiota-gut-brain axis is a critical component for GI and neurological functionality, and disturbances in one component can easily affect the other components. The host interacts with its microbiota through the release of neurotransmitters or other mediators in the gut, shifts in inflammatory status, and gut environment that can directly or indirectly impact the composition of the microbiota. Concurrently, neurotransmitters or metabolites from the microbiota interact with neurons in the ENS or nerve endings of the PNS, modulate inflammatory cells in the gut, but also extend their influence up to the HPA axis or neurotransmitter turnover in the brain. In this way, the microbiota can influence neurodevelopment and behavior and lead to changes in anxiety, depression, and pain perception. In recent years, it has become more apparent that the presence of the gut microbiota is essential for epigenetic regulation in the gut-brain axis. Much of the most exciting work has come from GF animals that exhibit a dysregulated epigenome in the brain, that contribute to neurobehavioral alterations. Colonization of GF animals or supplementation with pre- or probiotics are proven strategies to ameliorate neurobehavioral defects likely through altering the epigenome. Animal models of adult stress or early life stress clearly indicate that epigenetic modifications play an important role in the gut-brain axis. Reversal of these epigenetic changes often rescues the phenotype. Other studies have shown that the same stressors change the composition of the gut microbiota. Again, supplementation with pre- or probiotics normalizes the gut microbiota composition and often attenuates the phenotype. However, evidence linking interventions in the epigenome in the brain and alterations in the microbiota, or vice versa, is still lacking. In order to achieve the optimal desired effects, evidence suggests that the microbiota need to be modulated during critical neurodevelopmental periods. Taken together, accumulating compelling evidence suggests that the gut microbiota form a gateway to alter the epigenetically mediated pathophysiology of gut-brain disorders.

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DECLARATION OF CONFLICTING INTERESTS

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