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The Relationship Between the Serotonin Metabolism, Gut-microbiota and the Gut-brain Axis

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Abstract: Background: Serotonin (5-HT) has a pleiotropic function in gastrointestinal, neurological/psychiatric and liver diseases. The aim of this review was to elucidate whether the gut-microbiota played a critical role in regulating peripheral serotonin levels.

Methods: We searched for relevant studies published in English using the PubMed database from 1993 to the present.

Results: Several studies suggested that alterations in the gut-microbiota may contribute to a modulation of serotonin signalling. The first indication regarded the changes in the composition of the commensal bacteria and the intestinal transit time caused by antibiotic treatment. The second indication regarded the changes in serotonin levels correlated to specific bacteria. The third indication regarded the fact that decreased serotonin transporter expression was associated with a shift in gut-microbiota from homeostasis to inflammatory type microbiota. Serotonin plays a key role in the regulation of visceral pain, secretion, and initiation of the peristaltic reflex; however, its altered levels are also detected in many different psychiatric disorders. Symptoms of some gastrointestinal functional disorders may be due to deregulation in central nervous system activity, dysregulation at the peripheral level (intestine), or a combination of both (brain-gut axis) by means of neuro-endocrine-immune stimuli. Moreover, several studies have demonstrated the profibrogenic role of 5-HT in the liver, showing that it works synergistically with platelet-derived growth factor in stimulating hepatic stellate cell proliferation.

Conclusion: Although the specific interaction mechanisms are still unclear, some studies have suggested that there is a correlation between the gut-microbiota, some gastrointestinal and liver diseases and the serotonin metabolism.

Keywords: Serotonin, gut-microbiota, gut-brain axis, fibrosis, serotonin transporter, serotonin receptors, inflammation.

1. INTRODUCTION

The most clinically important diseases associated with the serotonin metabolism are gastrointestinal disorders, such as Irritable Bowel Syndrome (IBS), and neurological/psychiatric and hepatic diseases. Serotonin (5-HT) plays a key role in the regulation of visceral pain and secretion and in the initiation of the peristaltic reflex; however, altered levels of serotonin have also been detected in many different psychiatric disorders such as anxiety, depression, obsessive compulsive disorder and phobias [1, 2]. Moreover, several evidences [3] have demonstrated the profibrogenic role of 5-HT in the liver, showing that it worked synergistically with Platelet-derived Growth Factor (PDGF) in stimulating Hepatic Stellate Cell (HSC) proliferation. Serotonin degradation contributed to steatohepatitis in rodent models due to increased levels of reactive oxygen species which were generated when serotonin was degraded by enzyme monoamine oxidase A [3].

In the last decade, great advances were made in understanding the Structure and Functions of the Serotonin Transporter (SERT), a protein which is primarily involved in the metabolism and in the maintenance of constant serotonin homeostatic levels [4]. Selective inhibitors of this transporter (Selective Serotonin Reuptake Inhibitors - SSRIs) have proven to be effective in the management of several psychiatric disturbances, and they are currently being used

for treating diseases, such as fibromyalgia, dyspepsia and IBS, in which 5-HT seems to play a key pathophysiological role [5-7].

In recent years, several reports have suggested a possible role of the SERT in the pathophysiology and/or clinical expression of various diseases [8].

In particular, the link existing between the brain and the gut (the brain-gut axis), and the growing clinical evidence indicating the efficacy of SSRIs [1, 9] in different functional digestive disorders, such as IBS, prompted some authors to investigate the possible involvement of serotonin and its receptors [10], the SERT and its polymorphisms in their pathophysiology [11-19]. However, to the best knowledge of the authors, no study has investigated the role of SSRIs in patients with concomitant chronic liver disease and functional digestive disorders. On the other hand, depression is a common disorder in patients with chronic hepatopathies, and certain SSRIs have been reported to be safe and effective in treating these patients. Chang *et al.* [20] studied a cohort of 1380 newly diagnosed Hepatitis B Virus (HBV)-infected patients taking SSRIs matched by age, gender, liver cirrhosis and index year with a cohort of HBV-infected patients without SSRI use. These two cohorts of patients were followed from 2000 to 2012 to identify the incidence of Hepatocellular Carcinoma (HCC). The incidence rate of HCC was 1.28 and 3.51 per 1000 person-years for SSRI and non-SSRI users, respectively. The authors suggested that SSRI use may possibly reduce the risk of HCC in HBV-infected patients in a dose-responsive manner. The results of these studies suggested that SSRIs were safe and effective in treating IBS patients with concomitant liver disease. However, SSRIs can increase bleeding time

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by inhibiting the uptake and storage of platelet serotonin; therefore, caution should be used in cirrhotic patients at high risk for bleeding.

Irritable bowel syndrome is currently defined as a disorder of Gut-Brain Interaction [21, 22]. In IBS patients, symptoms may be due to alterations in Central Nervous System (CNS) activity (top-down), or to a dysregulation at the periphery (bottom-up), or a combination of both [23]. Several stressors either at the CNS level (external stress) or at the intestinal level (enteric stress) can stimulate the brain-gut axis. In fact, sensitivity and gastrointestinal motility are both regulated by the central and the peripheral nervous systems via the complex interplay existing between the brain and the gut [23]. Several studies have shown a high prevalence of anxiety and mood disorders among IBS patients [2, 13, 24-26]. On the other hand, low grade inflammation in the intestine may cause the activation of cerebral activity by afferent fibres as a result of the release of interleukin-1 (IL-1), IL-6, tumour necrosis factor- α (TNF- α) [23].

Several studies have shown that the gut-microbiota plays a pivotal role in psychiatric disorders [27, 28], such as major depressive disorder [29], anxiety- and trauma-related disorders [30]. It has also been suggested that alterations in the gut-microbiota may contribute to a modulation of brain-gut signalling which could alter the cortical response by means of neuro-endocrine-immune stimuli [31]. These data were confirmed by high rates of comorbidity between gastrointestinal and psychiatric illnesses [32]. Recently, Cao *et al.* [33] found that mice receiving faecal microbiota transplants from patients with constipation had a reduced trend in intestinal peristalsis and abnormal defecation habits. The authors demonstrated that this process causes the upregulation of SERT expression in the colonic tissues of Caco-2 cells (a cell line which expresses the SERT and innate immunity receptors) associated with decreased 5-HT content. Moreover, this method induced a decreased abundance of *Clostridium*, *Lactobacillus*, *Desulfovibrio*, and *Methylobacterium* and an increased trend of *Bacteroides* and *Akkermansia*, and resulted in damage to the intestinal barrier. Therefore, this study demonstrated that intestinal dysbiosis may upregulate SERT expression and may induce chronic constipation.

Based on these premises, the aim of this review was to elucidate whether the gut-microbiota played a critical role in regulating peripheral serotonin levels and outflow.

2. SEROTONIN METABOLISM

Serotonin is a mediator, which regulates important functions both in the brain and in the gastrointestinal tract. The digestive tract contains approximately 95% of the body's 5-HT; 90% stored in enterochromaffin cells (ECs) which release the transmitter in response to mechanical or chemical stimuli [10, 17, 34]. Serotonin released in the blood stream is taken up by a specialised SERT and stored in the dense granules of the platelets [3].

Serotonin cannot cross the blood-brain barrier unlike its precursor; therefore, serotonin is synthesised de novo within the serotonergic neurons of the CNS [35].

Serotonin exerts its biological activity by means of interaction with distinct receptor subtypes, currently classified according to their structures, molecular mechanisms and pharmacological profiles. The majority of 5-HT receptor subtypes are widely expressed along the gut and regulate a variety of digestive functions [10]. Serotonin released from the ECs regulates the sensory, motor and secretory functions of the digestive tract via the intrinsic and extrinsic nervous system. Intrinsic innervation is based on the neurons of the Enteric Nervous System (ENS), residing in the myenteric and submucosal plexus whereas extrinsic innervation is provided by the parasympathetic and sympathetic nervous systems. The ENS can work independently of the CNS, owing to the presence of Intrinsic Primary Afferent Neurons (IPANs) in the bowel [34].

Extrinsic enteric innervation is bidirectional; the bowel can affect the brain by means of the extrinsic primary afferent neurons located in the cranial nerves and dorsal root ganglia and the brain, in turn, can have an effect on the functions of the ENS via the parasympathetic nervous system which are coordinated with the local neuronal activity of ENS. In particular, the peristaltic and secretory reflexes are regulated by the release of 5-HT from the ECs and depend on the stimulation of 5-HT_{1P} and 5-HT₄ receptors on submucosal IPANs while 5-HT₃ receptors on IPANs may play a role in the initiation of other types of intestinal reflexes. In addition, post-synaptic 5-HT₃ receptors are present in both plexuses, including the motor neurons which innervate smooth muscle [34]. Serotonin can induce the release of acetylcholine by cholinergic neurons, producing smooth muscle contraction. It can also stimulate nitrergic neurons to release nitric oxide causing smooth muscle relaxation [17]. A main function of 5-HT₃ receptors is the activation of extrinsic sensory neurons which cause the pain and bloating associated with IBS.

The molecular and pharmacological features of the different 5-HT receptors as well as their implications in the control of the digestive functions and pathophysiology of IBS have been exhaustively addressed in previous reviews [10]. At present, drugs acting on 5-HT receptors are currently being used or investigated for increasing or decreasing colonic motility as well as visceral sensation and secretion in IBS patients [36].

The role of 5-HT in IBS is being extensively investigated [17, 37], and a number of studies have investigated the involvement of the SERT in the pathophysiology of this syndrome [11-16, 18, 19, 38].

Serotonin is also involved in liver tissue repair after ischemia/reperfusion injury. In fact, the expression of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{3A} and 5-HT_{3B} receptors has been identified in naïve mouse livers and the mRNA expression of 5-HT_{2A} and 2B receptors, which are well known to mediate mitogenic and developmental effects. Serotonin has been shown to increase after partial hepatectomy while its antagonists inhibited liver regeneration [35].

Platelets are the major source of sinusoidal serotonin during liver injury, and they are able to adhere to sinusoidal endothelial cells and also to translocate to the space of Disse where serotonin occurs in response to various proinflammatory mediators. Serotonin within the space of Disse is free to bind 5-HT receptors expressed by hepatocytes, both quiescent and activated HSCs. Serotonin has also been shown to regulate sinusoidal blood flow by 5-HT receptors expressed on endothelial cells and HSCs [39].

The serotonin transporter belongs to the family of the transporters of biogenic amines (including transporters for dopamine, noradrenaline, amino acid and osmolyte) [4, 40]. These are membrane proteins which transfer the substrate into the cell. The SERT is mainly related to the catecholamine transporters [4], and it is classified as the neuronal membrane sodium⁺/potassium⁺ (Na⁺/K⁺) dependent transporter [40]. The human SERT gene is mapped to chromosome 17q11.1-q12 [41], and the genetic locus for SERT, SLC6A4, is close to 17q12 [42].

Sequence analysis of DNA identified a 630-amino acid protein which crossed the membrane 12 times [40, 43]. Previous studies have suggested that SERT transmembrane domains probably contain aminoacidic residues associated with the binding site for 5-HT. In particular, the residues of the first and third transmembrane domains have been indicated as major determinants of binding with the substrate [44].

The 5-HT is involved in SERT regulation influencing activity and cell surface expression [45]. The SERT is expressed in several tissues at both the central and the peripheral level, such as the cell membrane of intestinal mast-cells [46], enteric serotonergic neurons and ECs [47], platelet membranes [48], lung [49], placenta [50],

hepatocyte, bile duct epithelial cell, HSCs [39]. The SERT controls the endogenous levels of 5-HT acting on 5-HT reuptake which is associated with the co-transport of both Na^+ and chloride (Cl^-) ions and a counter transport of K^+ . The intracellular reuptake of 5-HT can be blocked by fluoxetine and other SSRIs [51-53]. Moreover, in preclinical models it has been shown that the inhibition of digestive mucosal 5-HT uptake by SERT antagonists may enhance the responses of submucosal neurons to mechanical stimulation of the intestinal mucosa [51].

Hepatic stellate cells are also able to store 5-HT in an internal compartment from which it can be released, thereby initiating a possible feedback loop sustaining both hepatocyte and activated HSC proliferation. Both cell types are known to contribute to the regeneration of the hepatic mass following liver injury [35].

As regards the gastrointestinal tract, Bellini *et al.* [13] investigating the pharmacological characteristics of platelet SERT showed that SERT density and affinity were significantly decreased in IBS with diarrhoea (D-IBS) patients in comparison with healthy volunteers.

3. GUT-MICROBIOTA AND SEROTONIN METABOLISM

The gut is colonised by several microbes which may affect its motility and function. Intestinal microbiota is defined as the complex of microorganisms residing in the gut which maintain a symbiotic relationship with the host. The collective pool of genes of the microbes sharing this unique ecological niche is referred to as microbiome. This microbiota is not evenly distributed along the intestine, and the number of species increases from the esophagus to the rectum. The composition of the intestinal microbiota is very dynamic, and it changes from birth to adult life. It includes bacteria, archaea, viruses and protozoa. Bacteria can be classified into different phyla, orders, families, genera and species. Over 90% of the bacterial microbiota belong to two phyla, *i.e.* Firmicutes and Bacteroidetes, followed by Actinobacteria and Verrucomicrobia. During adult life, many conditions can influence the composition of the intestinal microbiota and changes in microbiota can affect health status or disease status. In fact, "normal" intestinal microbiota has been shown to confer different physiological benefits for the host, such as development of the immune system, protection against pathogens, regulation of intestinal homeostasis, and metabolic and neuroendocrine functions. The change in the intestinal microbiota is called dysbiosis, and is recognised as a predisposing factor for the development and progression of many chronic diseases, in particular those related to metabolic alterations including non-alcoholic fatty liver disease (NAFLD) [54].

Moreover, the gut-microbiota contributes to the regulation of 5-HT levels; 5-HT levels are decreased in germ-free (GF) mice as compared to specific pathogen-free (SPF) controls [55, 56]. No difference in the abundance of chromogranin A positive (CgA+) enteroendocrine cells was found between adult GF and SPF mice. Recently, Yano *et al.* [57] explored host-microbial interrelationships which regulate peripheral 5-HT by analysing microbial influences on the faecal metabolome. They found that GF mice exhibit notably reduced levels of colonic and faecal 5-HT in comparison with specific pathogen-free controls. This deficit in 5-HT was found along the distal, medial and proximal colon, but not in the small intestine, suggesting a specific role for the microbiota in regulating colonic 5-HT. In the study of Yano *et al.* no difference was found between adult GF and SPF mice in the abundance of chromogranin A (CgA)+ ECs, indicating that reduction in colon 5-HT may depend on an abnormal 5-HT metabolism rather than on the impaired development of ECs.

A recent study [58] has evaluated the influence of the depletion of gut microbiota obtained by antibiotic treatment on gut motility and analysed whether this effect was mediated by regulating the host serotonin metabolism. In this study, mice undergoing a 4-week oral administration of a combination of antibiotics showed modifi-

cations in the composition of commensal bacteria, and their transit time was slower. The treatment also induced inhibition of spontaneous phasic contractions of colonic longitudinal muscle. Antibiotic-treated mice also showed a decrease in colonic 5-HT whereas there was no difference in the abundance of CgA+ ECs when compared with SPF mice. However, intestinal ECs were morphologically larger in GF vs. SPF rats [59], suggesting that microbes may modify the development and/or function of ECs.

Hata *et al.* [60] hypothesised that bacterial enzymes in the gut lumen may contribute to free luminal 5-HT production by a deconjugation process. They measured luminal 5-HT levels in the gastrointestinal tract, and they analysed the contribution of gut microbes in the regulation of luminal 5-HT using GF mice and GF mice recolonised with SPF faecal flora (EX-GF). In this study, the faeces of male SPF mice were given orally to the GF mice. After 10 weeks, 5-HT luminal and tissue concentrations were determined and the expression levels of 5-HT-related genes were analysed in the ileum, caecum, and distal colon. The 5-HT levels were higher in the caecum and colon of EX-GF mice compared to the lumens of the GF mice whereas there was no significant difference in ileal 5-HT levels between the groups. In EX-GF mice, the luminal 5-HT concentration was higher in the colon as compared to the other parts of the gut. Tissue 5-HT concentrations in the colon were notably higher in the EX-GF mice as compared with the GF mice; nevertheless, there were no notable differences in either cecal or ileal tissue 5-HT concentrations between the groups. Analysis of the time course of the 5-HT production showed a rapid elevation in luminal 5-HT levels in the caecum and the colon after microbiota transplantation whereas a small and transient increase in 5-HT levels was found in the ileal lumen 7 days after the GF mice were exposed to SPF faeces. No significant changes were observed in the 5-HT tissue contents during the observation period.

Several studies have indicated that diets with Chlorogenic Acid (CGA) can alter the microbial composition of the gastrointestinal tract. Chlorogenic acid has antibacterial, antioxidant and anti-inflammatory properties, and it is one of the major dietary polyphenols present in various foods and beverages.

Wu *et al.* [61] investigated the effects of CGA on the regulation of the gut microbiota, using a 16 S rRNA gene-targeted metagenomic approach, and on the levels of tryptophan and 5-HT. Ninety-six healthy growing pigs were assigned to either standard feed plus 0.05% 3-caffeoylquinic Acid (3-CQA) CGA or a control group, and were observed for 60 days.

The majority of the high-abundance gut microbes in the CGA group were similar to those found in the control group but the diversity of the gut microbiota was increased after CGA supplementation. Absorption of aspartic acid, threonine, alanine and arginine was significantly increased in the CGA group as compared with the control group.

These data suggested that dietary CGA could change the 5-HT level in the colon, indicating the specific role of dietary CGA in regulating colonic 5-HT.

Bhattarai *et al.* [62] analysed serotonin-sensitive ion secretion in GF mice in comparison with mice colonised with human gut microbiota, and showed that GF mice had a notably greater secretory response to serotonin than their human gut microbiota controls, indicating that the presence of gut microbiota may modulate the secretory response to 5-HT.

Recent data have confirmed that formula feeding as compared to breast-feeding alters the colonic microbiota and may shift the tryptophan metabolism from serotonin to tryptamine, which may then lead to greater histamine levels and the risk of allergies in infants.

Saraf *et al.* [63] investigated, in a pig model, the effects of the formula diet as compared to breast-feeding on the colon and their influence on colon morphology and immune response and metabo-

lites implicated in the serotonin outflow and serotonin metabolism in the colon. The results of this study showed that the formula diet affected microbiota diversity and relative abundance in the piglet colon, with a reduction in sample richness in the formula group at all taxonomical levels, except at the order level. *Streptococcus*, *Blautia*, *Citrobacter*, *Butyrivimonas*, *Parabacteroides* and *Lactococcus* were all detected genera which were augmented with soy or milk formula feeding relative to sow feeding. *Butyrivimonas* and *Parabacteroides* levels were at least five-fold higher in formula-fed animals. These results indicated that the neonatal diet shaped the colon microbiota. Correlation analyses showed an important positive correlation of apoptosis with *Bacteroidetes* across diets. *Akkermansia*, *Butyrivimonas*, *Clostridium*, *Holdemania*, and *Bilophila* were positively correlated with the tryptophan metabolism in the soy-fed piglets while only *Bilophila* genus was associated with tryptophan metabolism in the milk group. To understand the impact of the formula diet on serotonin synthesis, the authors determined colon tissue serotonin concentrations, EC cells, 5HT-producing cells and serotonin transporter (vesicular monoamine transporter 1 - VMAT1) expression. Based on microbiota prediction analyses, Saraf *et al.* [63] found several genera which were associated with the tryptophan metabolism and demonstrated that formula diets suppressed the host serotonin level in the colons of piglets. No differences were found in serum serotonin levels, indicating that the small intestine (and other tissues) were involved in the serum serotonin levels.

An extensive review regarding therapeutic strategies concerning IBS [64] confirmed that probiotics are an attractive option in terms of both efficacy and safety in patients with IBS while a faecal transplant could represent a new therapeutic option for patients with an altered microbiota, although further studies are needed.

Choi *et al.* [65] demonstrated that the portal blood serotonin concentrations are selectively increased by high-fat diet feeding in mice, and they have endocrine effects on the liver. In fact, nutrient infusion induces 5-HT production in the human gut while the liver is the first organ to encounter gut-derived serotonin *via* the portal vein. Both gut-specific Tryptophan hydroxylase 1 (Tph1) knockout mice and liver-specific 5-HT 2A receptor knockout mice are resistant to high-fat diet -induced hepatic steatosis, without affecting systemic energy homeostasis. Moreover, selective 5-HT_{2A} receptor antagonist treatment prevented high-fat diet-induced hepatic steatosis.

According to this study, Haub *et al.* [66] demonstrated that tropisetron and palonosetron are effective in attenuating NAFLD in a genetic mouse model of obesity. This effect was associated with a marked decrease in the intestinal translocation of bacterial endotoxin, probably due to the stabilising effect of tropisetron and palonosetron (5-HT₃ antagonists) on the intestinal barrier integrity with a subsequent reduction in liver inflammation and fat accumulation.

4. GUT MICROBIOTA AND SERT

Gut microbiota may also influence intestinal homeostasis of 5-HT by affecting the 5-HT transporter (SLC6A4, mouse ortholog of human SERT) and/or 5-HT receptor expression (Fig. 1). Yano *et al.* [57] showed that a germ-free colon exhibited decreased expression of non-neuronal tryptophan TPH1. Germ-free mice also had elevated colonic expression of the 5-HT transporter SLC6A4, synthesised by enterocytes to favour 5-HT uptake. This may represent a compensatory response to a deficient 5-HT synthesis by host ECs since chemical tryptophan inhibition may modulate SLC6A4 expression.

Despite deficient levels of faecal and serum 5-HT, germ-free mice exhibited significantly increased levels of the tryptophan substrate, in both faeces and serum, indicating that primary disruption in host TPH1 expression may induce tryptophan accumulation.

Yano *et al.* [57] also demonstrated that postnatal reconstitution of the gut microbiota can correct the 5-HT deficiency seen in GF mice. Spore-forming bacteria colonization of GF mice increases 5-HT staining in CgA+ ECs, promotes host colonic TPH1 expression and decreases SLC6A4 expression with respect to levels seen in SPF mice. Amelioration in serum 5-HT is observed within 2 days after inoculation of GF mice with spore-forming bacteria. The authors also evaluated the outcomes of microbiota changes on colon and serum 5-HT in SLC6A4 heterozygous and complete knockout mice. Destroying the microbiota by antibiotic treatment of SPF SLC6A4 heterozygous and knockout mice efficiently reduced colonic 5-HT levels, suggesting that the microbiota was necessary for supporting gut 5-HT in SLC6A4-deficient mice. Antibiotic-induced reduction and spore-forming bacteria-induced increments in colon 5-HT levels may be ascribed to changes in the 5-HT content in colonic ECs from SLC6A4 heterozygous and knockout mice. These results suggested a role for spore-forming bacteria in supporting Tph1-mediated 5-HT biosynthesis by colonic ECs, adjusting both the colon and the serum levels of 5-HT.

The gene expression of 5-HT and associated molecules in the colon were also investigated by Hata *et al.* [60]. They found that the *Tph1* and *Slc6a4* expression levels in the submucous-mucous layer were efficiently reduced on day 3 after administration of SPF faeces in comparison with the basal levels; however, they reverted to basal levels at 21 days after the administration of SPF faeces. In the smooth muscle layer, the *5-Htr3a* mRNA levels were notably increased at 21 days after SPF faecal inoculation as compared with basal levels. Nevertheless, there was no relevant variation in *5-Htr4* mRNA levels before and after SPF faecal exposure.

Hata *et al.* [60] also showed that the total 5-HT levels in the colonic lumen of the EX-GF mice were twice as high as those of the GF mice, with approximately 90% of the 5-HT in the EX-GF mice found in an unconjugated (free) form while more than 50% of the 5-HT was found in a glucuronide-conjugated form in the GF mice. These results may suggest that gut microbes play a critical role in supporting luminal 5-HT levels. Moreover, the deconjugation process of glucuronide-conjugated 5-HT by bacterial enzymes can support the increased output of free 5-HT in the gut lumen.

Reigstad *et al.* [67] found that transcription of the *Slc6a4* gene is sex-dependent in the colonised colons of mice; decreased expression of *Slc6a4* was found in female mice. Both the genders of the host and gut microbes can affect *Slc6a4* and 5-HT expression in the gut. El Aidy *et al.* [68] investigated whether a lower 5-HT transporter expression has a relationship with a modified microbiome composition, analysing the 5-HT transporter in male and female wild types, heterozygous, and knockout 5-HT transporter rats maternally separated for 6 h a day from postnatal day 2 to day 15. Maternal separation was associated with a shift in the ratio between the two main bacterial phyla as defined by a reduction in *Bacteroidetes* and an increase in *Firmicutes*.

Layunta *et al.* [69] studied the consequences of Nucleotide Binding Oligomerization Domain Containing 1 (NOD1) activation on SERT activity and expression, and the interaction between NOD1 and other immune receptors (toll-like receptor 2 and toll-like receptor 4) in human enterocyte-like Caco-2/TC7 cells (a cell line which expresses the SERT as well as innate immunity receptors). The cytosolic receptor NOD1, highly expressed in the intestinal epithelial cells, plays a critical role in sustaining gut homeostasis, activating the innate immune system based on the bacteria or antigens present in the cytoplasm. The results of this study showed that NOD1 activation by its agonist (C-12-iEDAP-DAP) on SERT activity decreased the 5-HT uptake. Given that NOD1 activation decreased SERT activity, Layunta *et al.* [69] also investigated the impact of NOD1 on SERT molecular expression in Caco-2/TC7 cells, and they found that NOD1 activation inhibited SERT activity in Caco-2/TC7 cells and induced a notable reduction of SERT molecular expression. The intracellular signaling outflow involved was

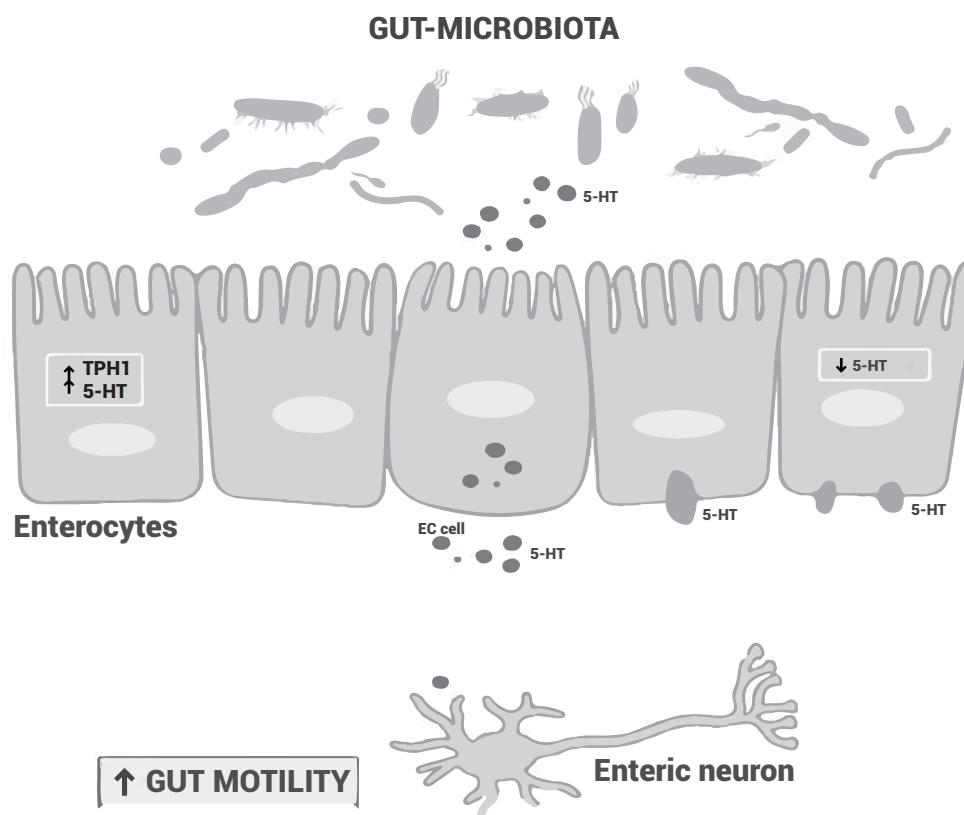


Fig. (1). Schematic view of the interactions between gut-microbiota and serotonin. The presence of specific bacteria of the gut-microbiota causes increased levels of non-neural tryptophan hydroxylase and subsequently of 5-HT production. Elevated levels of 5-HT also induce a decrease in 5-HT transporter levels.

also studied, showing that ERK intracellular signaling outflow was involved in NOD1 inhibitory control on the SERT. The SERT inhibition could induce increased 5-HT extracellular availability which was found to be a common factor in Inflammatory Bowel Diseases (IBD). The results of this study also showed that both NOD1 mRNA expression and NOD1 protein expression were preponderantly decreased in cells treated with 5-HT. Therefore, this study demonstrated that NOD1 activation reduces SERT activity and molecular expression, induced by the ERK/MAPK intracellular signaling outflow. This inhibitory control on SERT activity could increase the extracellular 5-HT availability, with consequently increased NOD1 expression, inducing a positive regulatory response.

The separation between wild type, heterozygous, and knockout serotonin transporter rats showed a significant difference in microbiota composition. The study of El Aidy *et al.* [68] showed that the 5-HT transporter genotype, especially when combined with early life stress, such as maternal separation, induced a state of microbiota dysbiosis. In fact, maternal separation in rats with decreased expression of 5-HT transporter caused the shift of gut-microbiota from homeostasis to inflammatory type microbiota defined by the elevated presence of *Desulfovibrio*, *Mucispirillum*, and *Fusobacterium*, all of which were correlated with a state of intestinal inflammation, including inflammation related to maternal separation and brain disorders, such as depressive disorders. In fact, in humans, the SERT is considered to be an excellent modulator of serotonergic neurotransmission in the central nervous system, and it is abundantly expressed in the cortical and limbic areas of the brain, which are involved in the emotional aspects of behaviour [70].

5. SEROTONIN, GUT-MICROBIOTA AND GUT-BRAIN AXIS

Luna *et al.* [71] investigated whether microbiome-neuroimmune profiles diverged in children with autism spectrum disorder and functional gastrointestinal disorders as compared with

neurotypical children. They found an increase in mucosa-associated Clostridiales in children with autism spectrum disorder and functional gastrointestinal disorders with a marked decrease in *Dorea*, *Blautia* and *Sutterella*. Tryptophan levels identified in the supernatant of the gastrointestinal biopsy specimens from the autism spectrum disorder and functional gastrointestinal disorders group were found to be significantly decreased when compared to either neurotypical patients with a functional gastrointestinal disorder or the neurotypical patients without functional gastrointestinal disorders. Moreover, serotonin pathway, 5-hydroxy-indole acetic acid (5-HIAA) (the primary metabolite of serotonin) was increased in patients with autism spectrum disorder with functional gastrointestinal disorders and in neurotypical patients with a functional gastrointestinal disorder as compared to neurotypical patients without a functional gastrointestinal disorder. Several organisms, in particular, *Erysipelotrichaceae*, *C. lituseburens*, and *Terrisporobacter* species correlate with tryptophan concentrations quantitated in the supernatant of the intestinal biopsy specimens from autism spectrum disorder patients with functional gastrointestinal disorders. Specific bacteria, cytokines and serotonergic metabolites correlate with gastrointestinal symptoms. It has been shown that several molecules with neuroactive functions, such as gamma-aminobutyric acid, catecholamines acetylcholine and serotonin, may be released from bacteria within the human gut [72]. These neurotransmitters are capable of interacting with the enteric nervous system and central nervous system. In particular, Thomas *et al.* [73] found that *L. reuteri* converts a dietary component, L-histidine, into an immunoregulatory signal, histamine, which suppresses pro-inflammatory TNF production. Members of the genera *Candida*, *Streptococcus*, *Escherichia* and *Enterococcus* synthesize 5-HT.

Golubeva *et al.* [74] investigated the interactions between intestinal microbiota, gut physiology and social behaviour in a BTBR T+ Itpr3tf/J mouse (one of the most widely used models of autism spectrum disorder). Similar to humans, these animals showed al-

terations in social interactions and enhanced engagement in repetitive behaviour. Golubeva *et al.* [74] studied the intestinal transit and the 5-HT tissue levels in the intestine of BTBR mice. They found a delayed transit time, probably due to the availability of 5-HT, which was found to be significantly reduced in the intestines of BTBR mice and associated with a downregulation of Tph1 and an upregulation of SERT gene expression. Golubeva *et al.* [74] also found that both the 5-HT tissue levels and the intestinal transit rate were closely associated with the relative abundance of *Firmicutes* bacteria. The reduced abundance of the *Rikenella*, *Parabacteroides*, *Odoribacter*, *Desulfovibrio*, *Blautia* and *Bifidobacterium* species was positively associated with the social behaviour scores and negatively associated with the repetitive behaviour and anxiety phenotype. The authors suggested that microbiota-based interventions in autism spectrum disorder can improve gastrointestinal and behavioural symptomatology in the BTBR mouse model of autism.

The interactions between gut-microbiota and the CNS are bidirectional; in fact, the brain can affect the composition of the microbiota as well as food preferences and appetite by means of the specific release of neurohormones into the gut lumen [75]. Moreover, the levels of the precursor of 5-HT (tryptophan), and the precursor of dopamine and noradrenaline (tyrosine) in the brains of germ-free mice are lower than in mice with recolonised gut microbiota [76]. On the contrary, in the hippocampus of germ-free mice, the 5-HT level and its main metabolite 5-hydroxyindoleacetic acid are higher than in colonised mice [77]. Colonisation of the germ-free animals restores peripheral tryptophan concentrations but it is inadequate in rearranging the modifications in hippocampal 5-HT levels [77].

Some studies have shown that lipopolysaccharide (LPS), which is an essential component of the outer membrane of gram negative bacteria, regulates plasma acute phase proteins and various other acute phase proteins involved in protein aggregation (A β aggregation). Moreover, LPS activates toll-like receptor 4 in mouse and human astrocytes, with subsequent neuroinflammation and neuron death. Martins [78] suggested that plasma LPS should be monitored in early life to prevent mitochondrial apoptosis in neurodegenerative diseases. Mota *et al.* [79] investigated the relationship between central 5-HT and LPS-induced systemic inflammation in animals. In this study, the authors showed a marked decrease in both the Anteroventral Preoptic Region (AVPO) 5-HT and 5-HIAA 300 min after LPS administration and enhanced corticosterone concentrations 45 and 300 min after LPS administration. The AVPO of the hypothalamus is one of the important regions of the CNS involved in the serotonin cascade. It is partially composed of thermoregulatory neurons which mediate fever when activated by prostaglandin E₂. On the contrary, an intracerebroventricular injection of 10 mcg of 5-HT in the LPS-injected group reduced LPS-induced fever, plasma corticosterone, and plasma IL-6 release. Korte-Bouws *et al.* [80] showed that LPS-induced 5-HT metabolism is significantly increased in both the Nucleus Accumbens (NAc) and Medial Prefrontal Cortex (mPFC) of homozygous (SERT+/+) male rats whereas this increase is partly or completely abolished in heterozygous (SERT+/-) or knockout (SERT-/-) male rats, respectively. The results of these studies suggested that bacterial LPS released by the intestine could interfere with the efficacy of drugs that act on the 5-HT receptor, potentially inducing adverse effects.

Martins [81] suggested that LPS and mycotoxins could cause membrane lipid disturbances with effects on cholesterol interacting proteins, lipoprotein metabolism and membrane apo E/amyloid beta interactions relevant to hypercholesterolemia associated with neurological diseases.

Olanzapine is a second-generation antipsychotic medication but it causes weight gain as a prominent side effect, which is a significant risk factor for metabolic syndrome and cardiovascular disease. Patients with schizophrenia have cognitive disorders, which not only do not improve with olanzapine treatment, but can even be exacerbated by the treatment itself [82, 83]. Olanzapine binds 5-HT

2A and 5-HT 1A, and 5-HT 2C receptors with high affinity which explains its therapeutic effects [84]. Davey *et al.* studied the composition of the intestinal microbiota and they showed that after olanzapine treatment an altered microbiota profile was associated with body weight gain [85].

Kao *et al.* [86] evaluated the therapeutic effect of prebiotic B-GOS[®] during the olanzapine treatment. They found that the prebiotic B-GOS[®] attenuated weight gain in female rats without compromising the central pharmacological action of the drug, associated with an increased TNF α , which is a recognised appetite suppressant. The authors suggested that prebiotic fermentation by gut microbiota could also improve the metabolic health of psychiatric patients treated with atypical antipsychotics.

Recently, Quigley [87] hypothesised that gut inflammation contributes to neurodegenerative diseases by means of bacterial signals from the oral cavity, stomach (*Helicobacter pylori*), small intestine (small intestinal bacterial overgrowth, SIBO), or colon ("dysbiosis") which lead to a local immune response generating proinflammatory cytokines which, in turn, impair blood barrier function and initiate a neuroinflammatory response. These consequences are intensified by the pro-inflammatory effects of a poor diet and other environmental factors, and the immunological and physiological consequences of aging (such as reduced neurotransmitters and increased oxidative stress).

CONCLUSION

Data have suggested that there is a correlation between gut-microbiota and the serotonin metabolism, although the specific interaction mechanisms still remain to be studied. It is still unclear how gut-microbiota interacts with the serotonin metabolism and the SERT. Providing an answer to such a question may be useful for a more complete understanding of the mechanisms underlying some gastrointestinal disorders and optimisation of therapy.

The literature examining the relationship between the role of gut microbiota in the pathophysiology and the response to drugs in gastrointestinal and liver diseases is still scarce even if it is growing rapidly. Studies on larger samples are needed to clarify the possible intriguing relationship among gut-microbiota and the serotonin metabolism.

The gut-microbiome is probably capable of affecting the drug metabolism, and could clarify the inter-individual differences in response to treatment and the development of side effects [88, 89]. This interaction could be responsible for the efficacy or the lack of efficacy of some drug acting on different 5-HT receptor subtypes, or causing the onset of adverse effects or dangerous drug interactions.

In addition, the well-known association between SERT polymorphisms and psychiatric disorders and the comorbidity between IBS and psychiatric disorders need to be additionally investigated. The good response to SSRIs in these different diseases is not a simple accident and the gut-microbiota could affect the efficacy and the tolerability of these types of drugs in the therapy of IBS and probably of other gastrointestinal disorders.

LIST OF ABBREVIATIONS

IBS	=	Irritable Bowel Syndrome
PDGF	=	Platelet-derived Growth Factor
HSCs	=	Hepatic Stellate Cells
SERT	=	Serotonin Transporter
5-HT	=	Serotonin
SSRIs	=	Selective Serotonin Reuptake Inhibitors
HBV	=	Hepatitis B Virus
HCC	=	hepatocellular Carcinoma
CNS	=	Central Nervous System
IL	=	Interleukin

TNF-alfa = Tumour Necrosis Factor-alfa
 ECs = Enterochromaffin Cells
 ENS = Enteric Nervous System
 IPANs = Intrinsic Primary Afferent Neurons
 NA = Sodium
 K = Potassium
 CL = Chlorine
 D-IBS = IBS with Diarrhoea
 NAFLD = Non-Alcoholic Fatty Liver Disease
 GF = Germ-free Mice
 SPF = Specific Pathogen-free
 CgA+ = Chromogranin A Positive
 EX-GF = Recolonised with SPF Faecal Flora
 CGA = Chlorogenic Acid
 CQA = Caffeoylquinic Acid
 VMAT1 = Vesicular Monoamine Transporter 1
 Tph1 = Tryptophan Hydroxylase 1
 SLC6A4 = Mouse Ortholog Of Human SERT
 NOD1 = Nucleotide Binding Oligomerization Domain Containing 1
 ERK = Extra-cellular Signal-regulated Kinase
 MAPK = Mitogen-Activated Protein Kinase
 5-HIAA = 5-Hydroxy-indole Acetic Acid
 LPS = Lipopolysaccharide
 AVPO = Anteroventral Preoptic Region
 NAc = Nucleus Accumbens
 mPFC = Medial Pre-frontal Cortex
 SIBO = Small Intestinal Bacterial Overgrowth

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

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