

# Tryptophan Metabolites Along the Microbiota-Gut-Brain Axis: An Interkingdom Communication System Influencing the Gut in Health and Disease

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**ABSTRACT:** The 'microbiota-gut-brain axis' plays a fundamental role in maintaining host homeostasis, and different immune, hormonal, and neuronal signals participate to this interkingdom communication system between eukaryota and prokaryota. The essential aminoacid tryptophan, as a precursor of several molecules acting at the interface between the host and the microbiota, is fundamental in the modulation of this bidirectional communication axis. In the gut, tryptophan undergoes 3 major metabolic pathways, the 5-HT, kynurenine, and AhR ligand pathways, which may be directly or indirectly controlled by the saprophytic flora. The importance of tryptophan metabolites in the modulation of the gastrointestinal tract is suggested by several preclinical and clinical studies; however, a thorough revision of the available literature has not been accomplished yet. Thus, this review attempts to cover the major aspects on the role of tryptophan metabolites in host-microbiota cross-talk underlying regulation of gut functions in health conditions and during disease states, with particular attention to 2 major gastrointestinal diseases, such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), both characterized by psychiatric disorders. Research in this area opens the possibility to target tryptophan metabolism to ameliorate the knowledge on the pathogenesis of both diseases, as well as to discover new therapeutic strategies based either on conventional pharmacological approaches or on the use of pre- and probiotics to manipulate the microbial flora.

**KEYWORDS:** Microbiota-gut-brain axis, central nervous system, enteric nervous system, enteric microenvironment, tryptophan, kynurenine, dysbiosis, irritable bowel syndrome (IBS), inflammatory bowel disease (IBD)

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## Introduction

The 'gut-brain axis' represents a complex network of pathways interconnecting the gut and the brain, comprising the central nervous system (CNS), the sympathetic and parasympathetic branches of the autonomic nervous system (ANS), the enteric nervous system (ENS), as well as the wide array of cells in the gastrointestinal microenvironment.<sup>1,2</sup> In the last decades, accruing evidences have led to the hypothesis that the gut saprophytic microflora may participate to this bidirectional communication system giving rise to the concept of a microbiota-gut-brain axis.<sup>1,3,4</sup> Along these pathways, neuronal, immune, hormonal, and metabolic mechanisms which are generated by both eukaryote cells of the host and prokaryotes participate to an interkingdom communication system.<sup>1,4,5</sup> The microbiota may directly or indirectly affect the local production of metabolites, controlling metabolic functions, immunity responses, and the defence against pathogenic microorganisms in the gut, thus establishing a mutually beneficial relationship with the host. An exciting emerging issue is that the extension of this microbial influence to the CNS may contribute to the regulation of brain development and behaviour.<sup>6,7</sup> In this perspective, it is obvious that alterations in the symbiotic cross-talk between the

microbiota and the host may bear important consequences, underlying development of both gastrointestinal and brain disorders.<sup>1,7,8</sup>

The possibility to clarify the mechanisms controlling host homeostasis along the microbiota-gut-brain axis is thus fundamental and different molecular pathways are now explored. Among different potentially neuroactive molecules, those deriving from tryptophan metabolism are of outstanding neurobiological interest.<sup>9,10</sup> The importance of tryptophan metabolites in the modulation of the gastrointestinal function is, indeed, suggested by several preclinical and clinical studies; however, a thorough revision of the available literature has not been accomplished yet. Thus, this review attempts to cover the major aspects concerning the role of gut tryptophan metabolites in the maintenance of gut homeostasis in health conditions. We also consider preclinical and clinical studies examining the involvement of tryptophan metabolites in the generation of microbiota-gut-brain axis signalling underlying major gut disorders such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), both characterized by psychiatric disorders. Research in this field allows to target tryptophan-generated metabolic pathways for the discovery of new potential therapeutic tools addressed to the treatment of IBS and IBD, which are characterized by important CNS disorders.<sup>11,12</sup>

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## The Gut Microbiota

The gastrointestinal tract is harboured by a complex community of microbial species (bacteria, virus, archaea, fungi, and protozoa), defined as 'gut microbiota', which establishes a mutual interaction with the host body.<sup>13</sup> The cross-talk between the host and its microbiota is important for achieving and maintaining host homeostasis, as the saprophytic microflora plays a central role in regulating physiological functions associated with nutrition, immune system activation, and defence of the host.<sup>14</sup> The interaction between the microbiota and the host may be influenced by important changes in the microflora composition during the lifespan.<sup>4</sup> The earliest bacterial signature appears already during foetal stages as *Firmicutes*, *Tenericutes*, *Proteobacteria*, *Bacteroidetes*, and *Fusobacteria* phyla have been identified in the placenta.<sup>15</sup> At birth, the composition of the microflora depends on the delivery method, and during early postnatal-life, the microbial community may be modified by different nutritional factors such as human breast or formula milk feeding or weaning to solid food.<sup>16</sup> The establishment of a healthy and stable microbiota in the first 3 years of life is crucial for the host metabolism and immune and nervous system development.<sup>17,18</sup> In adulthood, the saprophytic microflora becomes less variable,<sup>19,20</sup> although genetics, sex, lifestyle, or antibiotics treatment may influence its composition, selecting more adapted bacterial species.<sup>21,22</sup> The more representative bacteria phyla of healthy human adult microflora are *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Verrucomicrobia*, even though the *Firmicutes/Bacteroidetes* ratios vary between individuals.<sup>17,23</sup> The distribution of the gut microbiota displays differences along the gastrointestinal tract with increasing heterogeneity of bacterial community from oral regions to the distal colon and from the mucosal to luminal layer. Physiological changes and the increased incidence of diseases, in particular gastrointestinal disorders, may drastically alter the microbial composition during ageing, with an increasing abundance in *Bacteroidetes* with respect to *Firmicutes*.<sup>24-27</sup>

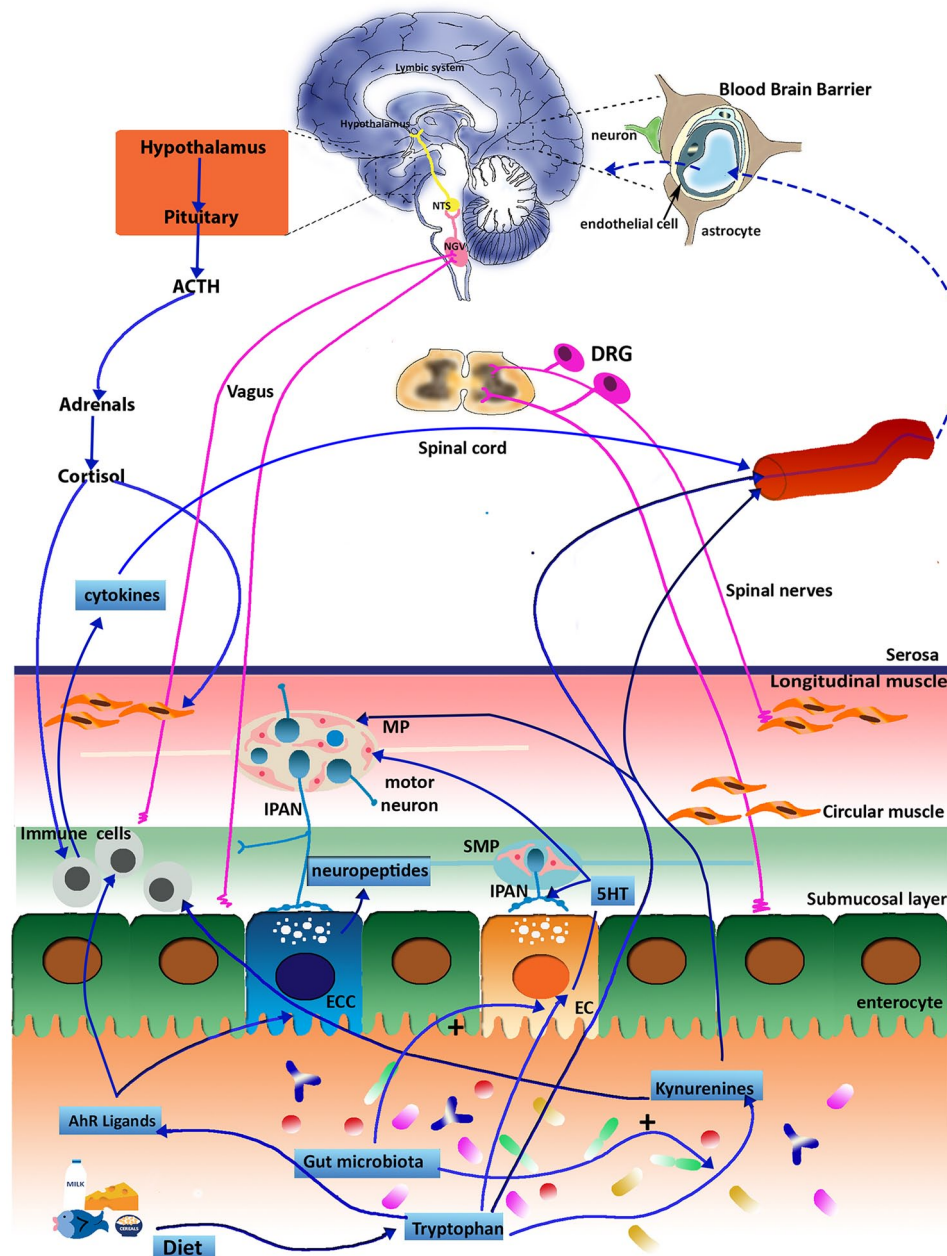
Accruing interest is emerging on the existence of an interkingdom communication system between the microbiota and the host. Bacteria harbouring the human gut produce many compounds impacting on the host, such as organic acids, bile salts, vitamins, gas, toxins, and virulence factors.<sup>28,29</sup> On the other side, the gut represents the best nutritional environment to allow bacterial growth. In this mutual interaction, while human molecules are well-known and characterized, prokaryotic products have been increasingly investigated as possible contributors to the regulation of several metabolic, immune, and nervous responses in the host.<sup>1</sup> Short-chain fatty acids (SCFAs) derived from the fermentation of dietary fibres in the colon,<sup>30</sup> bile acids produced in the liver and transformed in the gut lumen by the microbiota, and tryptophan metabolites exert different gastrointestinal and peripheral effects.<sup>1,31</sup> For example, bacterial metabolites such as SCFA, polyamine (ie, putrescine, spermidine, spermine), and aryl hydrocarbon receptor ligands, as well as molecular bacterial components, such as

lipopolysaccharide (LPS), lipoteichoic acid, peptidoglycan, flagellin, formyl peptides, and unique nucleic acid structures, influence the host immune responses.<sup>32</sup> It is now evident that the effect of bacterial components and metabolites, released at the gut level, may influence more distant sites, including the brain.<sup>4,6</sup> Furthermore, overabundance of virulence factors (ie, pigments, proteases, nuclease, toxins, haemophores) represents a family of bacterial molecules harmful for the host health.<sup>33</sup> This review focuses on the most recent studies regarding the role of tryptophan metabolites as an important emerging class of compounds involved in the host microbiota cross-talk, with a particular emphasis to their involvement in the regulation of gut functions in healthy conditions and during disease states, such as IBD and IBS.

## The Microbiota-Gut-Brain Axis

The existence of a gut-brain axis, allowing a constant cross-talk between the gut and the brain, both in health and disease conditions, via complex neuronal, hormonal, and immune reflexes has been proposed in the last few decades (Figure 1).<sup>34,35</sup> This bidirectional communication system drives sensory signals from the gut to the CNS, allowing the regulation of reflex activity and mood states. In turn, signals from the brain may influence motor, secretory, and immune gut functions.<sup>35,36</sup>

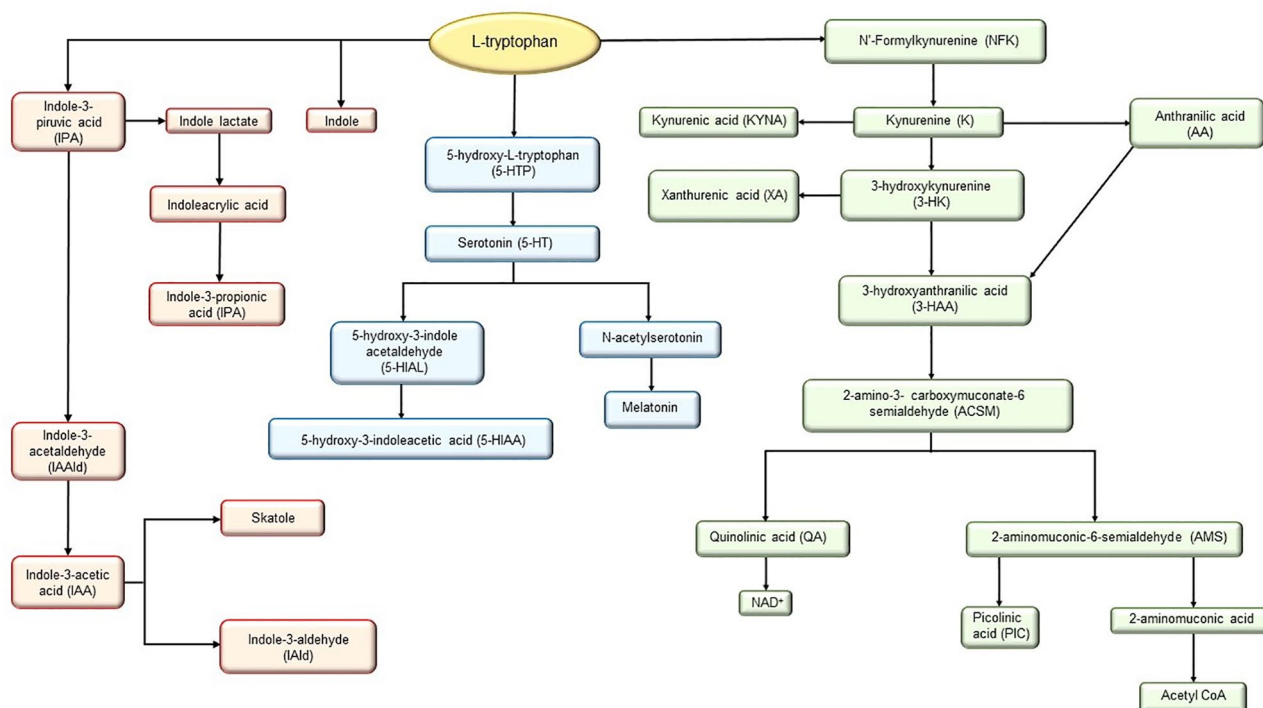
Afferent and efferent neuronal pathways, proceeding through the parasympathetic (vagal) and sympathetic (splanchnic and pelvic spinal pathways) branches of the ANS, represent the main neuronal connections. Vagal afferents, whose soma is present in the nodose vagal ganglion (NVG), transmit sensory information regarding the presence of food, motor activity, and degree of gut distension to the nucleus of the solitary tract (NTS) in the brain stem.<sup>36</sup> Neuronal inputs are then directed to higher CNS areas, for example, in the hypothalamus, or participate in long vago-vagal reflexes.<sup>36,37</sup> Efferent vagal signals, passing through the dorsal motor nucleus of the vagus (DMV), control gut motility and secretion.<sup>36,37</sup> Afferent spinal neurons of the sympathetic branch, whose soma are present in the dorsal root ganglia (DRG), transfer signals originating from the gut to secondary afferent neurons in the dorsal horn, which then project to the CNS via spinothalamic pathways, representing the main pain signalling pathways in the gut-brain axis.<sup>37</sup> Efferent sympathetic neurons have their cell bodies in the *tractus intermediolateralis* of the spinal cord, and synapse in the pre-vertebral ganglia with fibres impinging on vagal connections and on enteric neurons, to inhibit both gut secretion and motility.<sup>38</sup> Vagal and spinal afferent inputs synapse with higher brain regions, such as the emotional motor system, consisting in the limbic system and in some paralimbic structures (including the medial prefrontal cortex, amygdala, and hypothalamus).<sup>37,39</sup> At a peripheral level, the ENS, a complex neuronal network innervating the gastrointestinal tract, receives and transmits inputs to and from the ANS, representing a fundamental component of the bidirectional communication gut-brain axis.<sup>40,41</sup> An important hormonal player in the regulation



**Figure 1.** Schematic representation of the interplay occurring between Trp metabolites and different sections of the microbiota-gut-brain axis. The gut saprophytic microflora can signal to the CNS and to the ENS via endocrine, immune, metabolic, and neuronal pathways detailed in the text. In normal conditions, the blood-brain barrier allows the access of tryptophan and kynurenines, but not of circulating 5-HT, which is synthesized from tryptophan in the brain. ACTH indicates adrenocorticotrophin hormone; DRG, dorsal root ganglion; EC, enterochromaffin cells; ECC, enteroendocrine cell; IPAN, intrinsic primary afferent neurons; MP, myenteric plexus; NTS, nucleus of the solitary tract; NVG, nodose vagal ganglion; SCFA, short-chain fatty acid; SMP, submucosal plexus.  
Source: adapted from Baj et al.<sup>1</sup>

of several functions along the brain-gut axis is represented by the hypothalamic-pituitary adrenal (HPA) axis. Hypothalamic-pituitary adrenal axis activation is primed by the release of corticotrophin-releasing factor (CRF) from the hypothalamus, followed by the release of adrenocorticotrophin hormone (ACTH) from the pituitary, which then stimulates the adrenal glands to produce cortisol. The HPA axis is involved in the modulation of the gut motility, visceral sensation, and permeability, particularly during stress conditions.<sup>2,39</sup>

In more recent years, several preclinical and clinical studies have highlighted the fundamental influence that the enteric microbiota exerts on the gut-brain axis, which is now renamed 'microbiota-gut-brain axis'.<sup>35,42</sup> Although the exact mechanism/s of communication between the saprophytic microflora and the host have not yet completely understood, there are indications that microbes in the gut can directly stimulate afferent sensory neurons.<sup>43</sup> The probiotic *Lactobacillus reuteri* was shown to modulate gut motility as well as pain perception enhancing



**Figure 2.** Overview of tryptophan metabolism. Schematic representation of the major tryptophan metabolic routes yielding 5-HT, kynurenines, and indole derivatives/AhR pathways.

afferent excitability by the inhibition of calcium-dependent potassium channels.<sup>44</sup> Furthermore, alterations in the microbial composition and metabolomics have been correlated with altered neurotransmitter expression both in the CNS and in enteric neuronal pathways controlling gut sensory-motor functions.<sup>45,46</sup> A paradigmatic example is constituted by the ability of bacterial SCFAs to influence the production of different enteric neuropeptides by enteroendocrine cells (EECs), which, by diffusing through the lamina propria, gain access to the bloodstream and/or to local receptors, thus affecting intrinsic ENS neurons or extrinsic vagal innervation.<sup>47,48</sup> Furthermore, it is now ascertained that microbial metabolism may provide different precursors involved in biosynthetic pathways of classic neurotransmitters and/or neuromodulators, such as catecholamines (dopamine, noradrenaline, adrenaline), gamma amino butyric acid (GABA), and glutamic acid.<sup>1,49,50</sup> In addition, the gut microbiota, by direct or indirect control of tryptophan metabolism, contributes to the synthesis of a plethora of neuroactive molecules such as serotonin (5-HT), kynurenines, tryptamine, and indolic compounds, with important physiological roles in the control of gut-brain axis signaling<sup>9,10,51</sup> (Figure 1).

#### *Tryptophan metabolism along the microbiota-gut-brain axis*

Tryptophan is an aromatic amino acid containing an indolic group attached to an alanyl side chain. The amino acid is essential for animals and humans, which prevalently derive it from exogenous sources, such as dietary nutrient intake, and,

only in part, from endogenous protein degradation.<sup>52</sup> The main dietary sources of tryptophan are chocolate, cereals milk, milk derivatives, red meat, poultry, eggs, fish, and dried fruits. The free form of the amino acid is also contained in breast milk, playing an important role for the infant postnatal development.<sup>53</sup> The minimum daily requirement for adults is suggested to be about 250 to 425 mg a day.<sup>54</sup> In contrast to animals and humans, bacteria and plants produce high amounts of tryptophan from shikimic acid or anthranilate, and this ability has been exploited to obtain medically important indolic products.<sup>55</sup> The saprophytic microflora is not able to supply substantial amounts of tryptophan to humans, although some strains, such as *Escherichia coli*, were shown to produce the amino acid.<sup>10</sup> Tryptophan released from dietary proteins is absorbed through the intestinal epithelium and enter the blood circulation where it is present in an albumin-bound form and in a free form, this latter being fundamental for protein synthesis, thus sustaining body homeostasis and health.<sup>52</sup> In the gut, the amino acid may be also metabolized, under direct or indirect control by the microbiota,<sup>9,10</sup> giving origin to several compounds, such as 5-HT, kynurenines, tryptamine, and indolic compounds, which participate in the microbiota-gut-brain communication<sup>9,10,56,57</sup> (Figure 2). Degradation of tryptophan by the microflora appears not to be exclusively localized in the distal colon, where the bacterial proteolytic activity is highest, but occurs also in more oral regions of the gastrointestinal tract, as suggested for *Lactobacilli*-mediated tryptophan catabolism in the mouse stomach and ileum.<sup>58</sup>

*Kynurenine and its derivatives.* Under normal conditions, about 90% of the assumed tryptophan is catabolized and transformed into kynurenine through the kynurenine pathway, while approximately 3% is metabolized into 5-HT, and the remaining is degraded by the gut microbiota to produce indole and its derivatives.<sup>59</sup> The kynurenine pathway represents, therefore, the main tryptophan degradation pathway, leading to the formation of a large number of products (Figure 2). The biologically active form of the amino acid, L-tryptophan, is converted by either tryptophan 2,3-dioxygenase (TDO) or indoleamine 2,3-dioxygenase (IDO-1 or IDO-2) into N<sup>1</sup>-formyl kynurenine, which is further hydrolysed to kynurenine by N<sup>1</sup>-formyl kynurenine formamidase.<sup>60</sup> The expression of TDO and IDO is tissue and function specific, the first being mostly expressed in the liver,<sup>61</sup> while the second in extrahepatic tissues, including the intestine<sup>62</sup>; IDO-1 expression is enhanced by the presence of inflammatory mediators, such as interferon (IFN)- $\alpha$  and IFN- $\gamma$ , TNF- $\alpha$ , and LPS.<sup>63–65</sup> TDO expression is regulated at a transcriptional level by glucocorticoids such as cortisol, involving the *de novo* synthesis of the enzyme. In addition, TDO activity is also indirectly activated by tryptophan through the promotion of reactive oxygen species (ROS) production.<sup>66</sup>

Kynurenine can undergo further metabolic pathways yielding a plethora of biologically active molecules.<sup>49,67,68</sup> In normal conditions, kynurenine is hydroxylated by kynurenine hydroxylase to 3-hydroxykynurenine, which is further converted to 3-hydroxyanthranilic acid and, then, to 2-amino-3-carboxymuconate-6-semialdehyde (ACMS). ACMS is a highly reactive molecule that can undergo nonenzymatic cyclization to form quinolinic acid, which may further yield NAD<sup>+</sup>, an important end product of kynurenine.<sup>69</sup> Under specific conditions, ACMS may be enzymatically converted to 2-aminomuconic-6-semialdehyde that undergoes either nonenzymatic cyclization to form picolinic acid or enzymatic transformation to 2-aminomuconic acid, yielding acetylCoA.<sup>60</sup> Other branches of kynurenine metabolism lead to the production of either anthranilic acid or kynurenic acid by the enzymes kynureninase A and kynureninase aminotransferase, respectively. In normal conditions, the levels of these kynurenine are low but may raise under tryptophan or kynurenine loading and are influenced by vitamin B6 availability<sup>70</sup> (Figure 2).

From a functional view point, metabolites of the kynurenine pathway, collectively called kynurenines, in particular quinolinic acid and kynurenic acid, may influence neuronal activity both in the CNS and in the periphery, retaining neurotoxic and neuroprotective properties, respectively.<sup>67,71,72</sup> In this perspective, an unbalanced kynurenine metabolism has been suggested to underlay development of microbiota-gut-brain axis disorders.<sup>67,71,72</sup> In normal conditions, kynurenic acid and quinolinic acid do not cross the blood-brain barrier (BBB) in considerable amounts.<sup>73</sup> The BBB is, however, permeable to kynurenine, which is principally transformed into kynurenic acid in astrocytes and in quinolinic acid in the microglia. Quinolinic acid

may act as an excitotoxin both in the peripheral and CNS, due to its agonist properties at ionotropic glutamatergic N-methyl-D-aspartate (NMDA) receptors.<sup>74</sup> Quinolinic acid increases extracellular glutamate levels and acts synergistically with the amino acid to induce NMDA-mediated excitotoxicity.<sup>75–77</sup> The consequent intracellular Ca<sup>2+</sup> overload may trigger a cascade of dangerous events, including activation of protein kinases, phospholipases, nitric oxide synthase, protease, mitochondrial alterations, formation of ROS and reactive nitrogen species (RNS), lipid peroxidation, and cytoskeletal destabilization, ultimately leading to apoptotic or necrotic cell death.<sup>78</sup> A growing body of evidence suggests that quinolinic acid may participate in the pathophysiology of several psychiatric and neurodegenerative disorders.<sup>79–82</sup> In contrast, kynurenic acid is considered to be neuroprotective, acting as an antagonist at NMDA receptors both in the CNS and in the ENS.<sup>71,72,83</sup> In addition, kynurenic acid is also an antagonist at the  $\alpha$  nicotinic receptors and acts as an agonist with the G-protein-coupled receptor, GPR35.<sup>84,85</sup> The microbiota may affect host kynurenine production by influencing both the glucocorticoid-induced activity of TDO<sup>86</sup> and the immune system-dependent activity of IDO.<sup>87</sup> When the microbiota is completely absent, such as in germ-free (GF) rodents, or when the microflora composition is altered by antibiotic-induced dysbiosis, tryptophan plasma levels increase leading to a reduction in the kynurenine-to-tryptophan ratio, as a consequence of IDO and TDO activity changes.<sup>88–91</sup> Interestingly, the altered kynurenine-to-tryptophan ratio may be restored to normality after administration of probiotics such as *Bifidobacterium infantis*.<sup>91</sup> Microbiota-derived metabolites, such as SCFAs, affect IDO activity and kynurenine production,<sup>92</sup> while hydrogen peroxide produced by *Lactobacillus johnsonii* reduces circulating kynurenine levels in vivo in rats, as well as IDO activity in HT-29 intestinal epithelial cells.<sup>93,94</sup>

As the microbiota impacts on both the integrity of the BBB as well as on microglia and astrocytes maturation and function, an important issue, which remains to be clear-cut elucidated yet, concerns the ability of the saprophytic microflora to influence kynurenine synthesis in the CNS, particularly in the early developmental stages, as prenatal inhibition of kynurenine pathways induced changes of synaptic transmission and protein expression in the CNS which were associated with cognitive alterations in the adult offspring.<sup>95–97</sup>

*5-HT.* In humans, the gastrointestinal tract represents the main source of 5-HT (about 95% of all body sources) where the biogenic amine is released from enterochromaffin cells (ECs) of the mucosa and from myenteric neurons. In the gut, 5-HT is important for its hormonal and neuronal actions and is implicated in the control of mucosal secretion, absorption of nutrients, vasodilator, motor, and sensory functions, including perception of pain and nausea.<sup>98,99</sup> A small amount of tryptophan (3%) is used for the production of enteric 5-HT, which involves the activation of the key enzyme tryptophan

hydroxylase (TPH). Two homologous isoenzymes, TPH1 and TPH2, have been characterized, TPH1 is mostly expressed in the gut, while TPH2 is localized in the brain.<sup>100</sup> As the BBB is not permeable to the biogenic amine, TPH2 is fundamental for the brain synthesis of 5-HT from free circulating tryptophan.<sup>49,101</sup> In the CNS, 5-HT is involved in the modulation of mood, behaviour, and cognitive functions and changes in the serotonergic transmission underlay development of psychiatric disorders, including major depression and schizophrenia.<sup>102</sup> Further metabolic reactions downstream to 5-HT include its conversion by monoamine oxidase (MAO) into 5-hydroxy-3-indole acetaldehyde, which after successive enzymatic transformations yields melatonin<sup>103</sup> (Figure 2). 5-HT exerts its effects both in the gut and in the CNS via activation of serotonergic receptors subdivided into 7 families, from 5-HT<sub>1</sub> to 5-HT<sub>7</sub>, with 5HT<sub>3</sub> and 5HT<sub>4</sub> being the most represented in the gastrointestinal tract. Except for 5-HT<sub>3</sub> receptor, which is a ligand-gated Na<sup>+</sup> and K<sup>+</sup> cation channels, all these receptor families consist of metabotropic G protein-coupled receptors.<sup>104</sup>

The microbiota has been proposed to take part in the regulation of 5-HT gut levels. Bacterial strains, such as *Lactococcus lactis subsp. cremoris*, *Lactobacillus lactis subsp. lactis*, *Lactobacillus plantarum* (FI8595), *Streptococcus thermophilus*, *Escherichia coli* K-12, *Morganella morganii*, *Klebsiella pneumoniae*, and *Hafnia alvei*, were shown to produce 5-HT in vitro from tryptophan.<sup>105-107</sup> There are also studies suggesting that gut bacteria may influence in vivo 5HT gut levels by controlling the amine biosynthesis, metabolism, and transport.<sup>108-110</sup> In the lumen of the cecum and colon of GF mice, 5-HT levels were significantly lower than in mice colonized with specific pathogen free (SPF) or with faecal flora (ex-GF).<sup>109</sup> Recolonization was followed by an increase in the concentration of free unconjugated, biologically active 5-HT, suggesting that bacteria sustain the accumulation of free luminal 5-HT by promoting the deconjugation of glucuronide-conjugated 5-HT.<sup>109</sup> Microbiota metabolites concur to modulate 5-HT production in the gut. In ex-GF mice colonized with human gut microbiota, colonic TPH1 mRNA and protein levels as well as the amount of intraluminal 5-HT enhanced with respect to corresponding GF animals.<sup>108</sup> Incubation of a human cell line of EC cells with SCFAs enhanced the levels TPH1 mRNA.<sup>108</sup> In addition, microbiota-derived secondary bile acids have been shown to regulate 5-HT synthesis in the host via a G protein-coupled receptor, TGR5.<sup>111</sup> There are studies suggesting that microbiota-driven changes of 5-HT gut levels may influence the gut-brain communication. For example, in a transgenic mouse model of autism spectrum disorders, 5-HT gut levels were significantly lower than in the corresponding controls and significantly correlated with the abundance of bile-metabolizing *Bifidobacterium* and *Blautia*.<sup>112</sup>

*Microbiota-derived tryptophan metabolites.* The amount of intestinal tryptophan, which is not conveyed into the systemic circulation after absorption, may be locally transformed by

different gut microbial species into a variety of catabolites, via various metabolic pathways (Figure 2).<sup>58</sup> In the *Firmicutes* phylum, *Clostridium sporogenes* and *Ruminococcus gnavus* convert tryptophan into tryptamine, a biogenic amine structurally similar to 5-HT, by activation of tryptophan decarboxylases (TrpDs).<sup>113</sup> Interestingly, in GF mice, the gut levels of tryptamine are reduced suggesting that the microbiota is fundamental for tryptophan decarboxylation to tryptamine.<sup>114</sup> Although TrpD activity is remarkably rare among bacteria, metagenomic data demonstrate that at least 10% of the human population harbours at least one bacterium encoding the enzyme.<sup>113</sup> For example, tryptamine producing *Ruminococcus gnavus* is a common species of the gut microbiota found in around 90% of adults<sup>115</sup> and infants.<sup>116</sup> This is all the more interesting since tryptamine has an important role in maintaining gut homeostasis.<sup>58</sup>

The gut microbiota has also a fundamental role in the transformation of tryptophan into indole and its derivatives, such as indole-3-aldehyde (IAld), indole-3-acetic acid (IAA), indole-3-propionic acid (IPA), indole-3-acetaldehyde (IAAld), and indoleacrylic acid.<sup>58</sup> Indolic compounds represent inter-species and inter-kingdom class of signalling molecules, which contribute to control different aspects of bacterial physiology, such as sporulation, biofilm formation, and antibiotic resistance, and may also support maintenance of epithelium integrity and of the gut immune functions in the host.<sup>10</sup> Indolic compounds are produced by the enzyme tryptophanase (TnaA) expressed in a large number of microorganisms, including *Escherichia coli*, *Proteus vulgaris*, *Paracolobactrum coliforme*, *Achromobacter liquefaciens*, and *Bacteriodes spp.* *Escherichia coli*, and possesses 3 permeases for tryptophan transport and produces indole metabolites as by-products of the amino acid metabolism to obtain carbon and nitrogen.<sup>117</sup> In *Lactobacillus reuteri* and *Lactobacillus johnsonii*, an aromatic amino acid transferase catalyses the production of IAld,<sup>118</sup> while some *Lactobacillus*, *Bacteroides*, and *Clostridium* spp. convert IAA acid into skatole, which can affect the growth and reproduction of several bacteria, including *Salmonella*, *Shigella*, and *Escherichia*.<sup>119-121</sup> *Clostridia* are also able to catabolize tryptophan to indole pyruvic acid, which is then converted to IAA.<sup>122</sup> Indoles are natural ligands of the aryl hydrocarbon receptor AhR, a transcription factor with an important role in the maintenance of host homeostasis.<sup>123</sup> AhR is present in an inactive form in the cytoplasm and translocates into the nucleus after binding to its ligands, consisting in dietary components, xenobiotics, and bacterial metabolites.<sup>10,123</sup> In the nucleus, the activated form of AhR binds to the aryl hydrocarbon receptor nuclear translocator (ARNT) inducing the expression of genes containing specific DNA enhancer sequences, called aryl hydrocarbon response elements (AhREs), which codify for enzymes implicated in xenobiotic metabolism and in detoxification of carcinogenic polycyclic aromatic hydrocarbons.<sup>123</sup> The physiologic role of AhR in the host seems, however, to expand to the control of several other functions including the modulation of

epithelial cell turnover and the immune response immune at the gut level.<sup>124</sup>

The availability of methods for the determination of concentrations of microbial tryptophan catabolites in human biological specimens is rather limited; however, available data suggest that the indole is the most abundant microbial tryptophan catabolite, followed by IAA and IPA.<sup>125,126</sup> More efforts are needed to correlate the abundances of bacterial species with concentrations of tryptophan catabolites as well as to compare metabolite concentrations across biological compartments (ie, faeces, blood, and urine) and between different human populations.<sup>58</sup>

### The Interplay Between the Gut Microbiota and the Enteric Environment: Role of Tryptophan Metabolites

The following paragraphs will give a concise description of the main evidences demonstrating the physiological relevance of tryptophan metabolites in the interplay occurring between the gut microbiota and the enteric microenvironment. The enteric microenvironment is constituted by different cell types, including enterocytes, EECs, neurons, enteric glial cells, smooth muscle cells, and immune cells, which receive and send signals from and to the microbial flora (Figure 1).<sup>1</sup> Indeed, this cross-talk is fundamental in the regulation of the bidirectional communication along the microbiota-gut-brain axis.

#### Epithelial barrier

The monostratified intestinal epithelium is the widest mucosal surface in the human body, contributing to separate the host organism from the outer environment, thus supporting the host health. The epithelial barrier restricts the contact of luminal microbes with the underlying intestinal tissue by secreting a protective viscous mucus layer from specialized goblet cells. In addition, enterocytes express tight-junction proteins (such as occludin, junctional adhesion molecule, and claudin family members that interact with cytoplasmic linker proteins, such as zonula occludens-1, ZO-1) which strengthen the barrier function against bacteria and antigens contained in the lumen.<sup>127</sup> The gut microbiota supports the intestinal barrier function by favouring enterocyte proliferation, enhancement of epithelial cell integrity, via translocation of tight-junction proteins, and by upregulating gene expression involved in desmosome maintenance.<sup>23,128</sup> Germ-free (GF) animals display a reduced intestinal surface area, slower epithelial cell turnover, and increase EC cell area and smaller villous thickness, with respect to their conventional controls.<sup>129</sup> *Bifidobacterium* and *Lactobacillus* species enhance the survival of gut epithelial cells by inhibiting pro-apoptotic pathways associated with pathogenic bacteria.<sup>130-132</sup> Among the different microbiota-derived tryptophan metabolites, indolic compounds are relevant for the regulation of bacterial motility and for the formation of the biofilm, a multicellular structure generated through the

expression of extracellular adhesion factors, which prevents the invasion of non-indole producing pathogenic bacterial species, such as *Salmonella enterica* and *Pseudomonas aeruginosa*.<sup>133-136</sup> In *Lactobacillus reuteri* and *Lactobacillus johnsonii*, an aromatic amino acid transferase catalyses the production of IAld, which contributes to the maintenance of intestinal homeostasis by preventing the colonization of pathogenic microorganisms (such as *Candida albicans*) and by weakening inflammatory responses.<sup>118</sup> Both in vitro and in vivo studies have shown that indole increases the stability of the barrier functions by inducing the expression of genes involved in maintenance of epithelial cell structure and function.<sup>137,138</sup> Indole acrylic acid produced by different *Peptostreptococcus* spp may favour the epithelial barrier function and reduces inflammatory responses in mice by promoting goblet cell differentiation and mucus production, via AHR activation.<sup>139</sup> In GF mice colonized with either a wild-type bacterial community or fldC mutant *Clostridium sporogenes*, lacking the fldC gene encoding for the phenyllactate dehydratase essential for IPA production, the fldC-colonized mice exhibited significantly increased permeability to fluorescein isothiocyanate (FITC)-dextran compared to their wild-type-colonized counterparts, caused by the deficiency of luminal IPA.<sup>140</sup> In high-fat diet-fed mice, IPA was recently found to reduce intestinal permeability and displayed prominent efficacy in diminishing in vitro T84 cell monolayer permeability compromised by pro-inflammatory cytokines.<sup>141</sup> IPA promoted upregulated junctional protein-coding mRNA as well as downregulation of enterocyte TNF- $\alpha$  by directly engaging the X receptor (PXR) and both effects involved Toll-like receptor (TLRs) signalling in enterocytes.<sup>142</sup> TLRs constitute one of the best characterized family of pathogen-associated molecular pattern (PAMP) receptors.<sup>143</sup> Activation of TLRs promotes intracellular signals associated with distinctive adaptor proteins, such as myeloid differentiation primary response gene 88 (MyD88) and TIR-domain-containing adapter-inducing interferon- $\beta$  (TRIF) pathway. As a consequence, several downstream pathways, comprising the nuclear factor-kappa B (NF- $\kappa$ B), mitogen-activated protein kinases (MAPK), and/or interferon-regulatory factor (IRF) signalling pathways, are activated favouring the expression of several gene products.<sup>144,145</sup> Activation of TLRs by specific bacterial products, such as lipopeptides, peptidoglycans, and glycolipids, induce epithelial cell proliferation and surface repair following injury<sup>146</sup> and may reinforce the integrity of enterocytes sustaining the translocation of ZO-1.<sup>147</sup> Interestingly, PXR-deficient (Nr1i2<sup>-/-</sup>) mice displayed a distinctive 'leaky' gut physiology coupled with upregulation of TLR4 signalling pathway and these defects in the epithelial barrier were corrected in Nr1i2<sup>-/-</sup>Tlr4<sup>-/-</sup> mice.<sup>142</sup> Authors suggest that homeostasis between indole secreting bacteria, epithelial PXR, and TLR4 expression is required to prevent intestinal barrier dysfunction, as may occur in some pathological conditions, including IBS and IBD. In these conditions, microorganisms and/or of their metabolites by

crossing the epithelial barrier may directly interact with neurons and immune cells in the gut wall. The consequent release of neurotransmitters/neuromodulators as well as of pro-inflammatory cytokines may influence the gut function, but may also extend its action to more distal sites, reaching the CNS and predisposing to psychiatric disorders.<sup>148,149</sup> The cause/effect relationship between gut permeability changes and brain disorders still remains to be clearly defined; however, the possibility to re-establish a healthy epithelial barrier resorting to microbial-based approaches, including modulation of tryptophan metabolite signalling, may represent a possible adjuvant approach for the treatment of gut-brain disorders.<sup>150</sup>

**Enteroendocrine cells.** The epithelium of the gastrointestinal tract contains different types of single sensory/secretory EECs, which represent the largest endocrine system of the human body.<sup>151</sup> At least 15 types of EECs have been described, releasing more than 20 peptide hormones involved in the regulation of gut motility, gastric acid secretion, and metabolic and behavioural responses, such as food intake.<sup>152</sup> These hormones comprise 5-HT, chromogranin/secretogranin family, somatostatin, neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), substance P (SP), cholecystokinin (CKK), glucagon-like peptide (GLP)-1/2, and ghrelin.<sup>153,154</sup> The apical surface of enteroendocrine cells (ECCs) comes into contact with luminal constituents, including bacterial metabolites, and takes part in modulation of local neuronal and glial networks by releasing peptides and biological active molecules towards the intestinal submucosa.<sup>155,156</sup> Gut signalling molecules from EECs may influence also the brain function, via hormonal and humoral routes, thus participating in the gut-brain communication axis.<sup>42</sup> This interplay also extends to the gut microbiota as the saprophytic microflora has a modulatory role on the release of gut peptides from ECCs.<sup>157</sup> For example, type L cells ECCs respond to luminal nutrients secreting anorexigenic hormones involved in food consumption, such as postprandial release of GLP-1 and peptide YY (PYY).<sup>158-160</sup> Receptors for both hormones are located along the gut-brain axis, in the ENS, on vagal afferents, and in the brain stem and hypothalamus in the CNS.<sup>161-163</sup> The gut microbiota may contribute to gut chemosensitivity,<sup>48</sup> as L cells can sense diverse food components via different receptors located on the luminal apical border, including free fatty acid receptor types (FFAR1-4) for bacterial SCFAs.<sup>164</sup> Among tryptophan metabolites, indole has been described as a signalling molecule involved in the modulation of GLP-1 from immortalized Glutag L cells as well as from mouse colonic primary L cells.<sup>165</sup> Indole influences GLP-1 release in a dual modality, exerting a stimulatory effect over short time periods and an inhibitory effect after prolonged exposure. These data add further information on the ability of tryptophan to modulate appetite. It was in fact proposed that a high-protein diet rich in tryptophan content may suppress appetite via 5-HT production.<sup>166</sup>

ECs represent a class of EECs located in the epithelial layer of the whole gastrointestinal tract whose basolateral border is in contact with afferent and efferent nerve terminals located in the lamina propria.<sup>153,154</sup> ECs display a peculiar bidirectional signalling function from the microbiota to the gut and to the brain.<sup>4</sup> ECs secrete 5-HT and peptides (ie, corticotrophin-releasing hormone, CKK, and somatostatin) in response to various physiological and pathological luminal stimuli,<sup>153,154</sup> including microbial metabolites or bacterial toxins,<sup>167,168</sup> but may also respond to central stimuli.<sup>169</sup> The presence of pathogenic bacteria in the gut lumen, including *Escherichia coli*, *Vibrio cholerae*, and *Salmonella typhimurium*, was linked to upregulation of 5-HT secretion into the lamina propria.<sup>170</sup>

The gut microbiota influences several gut functions by regulating 5-HT biosynthesis, release, and metabolism in ECs. The mouse gut microbiota metabolite deoxycholate produced by indigenous spore-forming *Clostridium* species stimulated 5-HT biosynthesis in colonic ECs and gastrointestinal motility.<sup>171</sup> The saprophytic microflora modulates 5-HT release from ECs by controlling the activity of different receptors for neurotransmitters, hormones, and TLRs.<sup>172,173</sup> LPS stimulated the release of 5-HT in vitro from ECs of Crohn disease patients via TLR4 activation.<sup>174</sup> 5-HT released by ECs, in a paracrine way, activates its own receptors on intrinsic and extrinsic primary afferent nerve terminals laying in the proximity of ECs, consequently triggering enteric reflexes mediating peristalsis, secretion, pain perception, and inflammatory responses.<sup>98,175</sup> Secretomotor reflexes, underlying chloride ions and water release into the lumen, are promoted by strains of pathogenic bacteria.<sup>176</sup> The consequent enhancement of intraluminal fluid content favours bowel motility and the clearance of the intestinal content, including pathogenic bacteria. Although this issue has not yet been fully elucidated, 5-HT secreted by ECs is suggested to intervene in the interkingdom communication between the microbiota and the host by influencing bacterial motility and promote the expression of virulence genes.<sup>98,177-179</sup>

### *The gut-associated immune system*

The physiological maintenance of host tissue homeostasis depends on the equilibrium between adaptive and innate immunity that regulates responses to food intake, commensals, and pathogens. In the gut, complex interplaying mechanisms mediated by the gut-associated lymphoid tissue (GALT) are responsible for the activation of both innate and adaptive immunity.<sup>180</sup> The GALT represents the most extensive lymphoid system of the human body and comprises both inductive Peyer patches, composed of aggregated lymphoid tissue and considered as the immune sensors of the gut, and effector sites, such as lamina propria lymphocytes and intraepithelial lymphocytes (LPLs and IEL, respectively).<sup>181,182</sup> This complex intestinal immune system is essential for the development of oral tolerance to avoid inflammatory responses against food proteins and self-aggression against the resident intestinal



microbiota.<sup>183</sup> Dendritic cells (DC), placed below epithelial cells, are able to phagocytize antigens and bacteria and to expose cleaved fragments of processed antigen to major histocompatibility complex (MHC) class I or II molecules, and are the first immune cell type to modulate tolerance. Activation of DCs after the recognition of MHC molecules is necessary for the stimulation and expansion of CD4<sup>+</sup> helper T-cells and the lack of costimulatory signals on immature DC cell phenotype induces T cell oral tolerance. The continuous exposure to food and to microbiota antigens prevents the activation of DC cells, leading to a tolerogenic environment.<sup>184</sup> Intestinal naïve T-cells are classified into 2 classes of CD4<sup>+</sup> T-cells according to the array of cytokine produced: T helper type 1 (Th1) that modulates cell-mediated immunity and secrete INF $\gamma$  and TNF $\alpha$  and T helper type 2 (Th2) that modulates humoral immunity and secrete interleukin (IL)-4 and IL-6, IL-10, and TGF $\beta$ . Induction of Th2 response and downregulation of Th1 response has been suggested as a part of a physiological mechanism of oral tolerance.<sup>185</sup> Generation of a regulatory T cell subset (Treg) with suppressive effects may also favour development of oral tolerance.<sup>186</sup> The intestinal microflora with its qualitative differences in composition during lifespan may affect immune responses in the gut. Studies carried out on GF rodents revealed that the microbiota is essential for the development of the GALT, playing a vital role in shaping gut immune system homeostasis, with IgA secretion and controlled inflammation being considered as a consequence of bacterial colonization.<sup>187</sup> In comparison to conventionally housed animals, GF animals have fewer and smaller Peyer patch follicles, decreased levels of circulating plasma cells and IgA associated with a decreased expression of activation markers on intestinal macrophages, decreased MHCII on epithelial cells, and decreased nitric oxide and histamine levels in the small intestine.<sup>188-192</sup> Re-conventionalization of GF mice with a normal microflora re-establishes the mucosal immune system.<sup>193</sup> The balance between the microbiota, immune response, and tolerance mechanisms is fundamental for a healthy intestine and any alteration of this relationship may result in gut disorders, but may also extend more distally to the brain.<sup>4,194</sup> For example, after inducing water avoidance stress, B and T cell-deficient Rag1<sup>-/-</sup> mice displayed altered responses to memory and anxiety tests, with decreased hippocampal c-Fos expression, increased HPA-axis activation, and hypersecretory intestinal activity and dysbiosis.<sup>190</sup> All the peripheral and central dysfunctions were normalized by pre-treatment with probiotics, *Lactobacillus rhamnosus* and *Lactobacillus helveticus*, indicating that probiotics may ameliorate the immune-mediated alterations of the gut-brain-microbiota axis.<sup>190</sup> Metabolites deriving from the gut microbiota may have immunomodulatory properties. SCFA-producing bacteria of the genera *Clostridium* stimulate Treg cell expansion and differentiation.<sup>195</sup> In the kynurenine pathway, quinolinic, kynurenic, and picolinic acid are involved in the regulation of the gut immune function.<sup>196,197</sup> Kynurenic acid reduced

LPS-induced IL-23 expression in DCs and inhibited the in vitro differentiation of Th17 cell, a subset of pro-inflammatory Th cells, inducing anti-inflammatory effects.<sup>198</sup> Kynurenic acid may also blunt the secretion of high-mobility group box 1 protein, a fundamental mediator of inflammation, from monocytes as well as the secretion of  $\alpha$ -defensin, HNP 1-3, from cultures of granulocytes in vitro.<sup>199</sup> Kynurenic acid and newly synthesized analogues have an important role in suppressing TNF- $\alpha$  synthesis by influencing the expression of the anti-inflammatory factor TSG-6.<sup>200</sup> Kynurenic acid also inhibits xanthine oxydase in vitro, resulting in less oxygen species production.<sup>201</sup> In mice, in vivo treatment with kynurenic acid attenuated LPS-induced TNF- $\alpha$  and nitric oxide serum levels and reduced LPS-induced death.<sup>202</sup> Kynurenic acid was also able to reduce TNF- $\alpha$  release from leukocytes after ex vivo exposure to LPS.<sup>203</sup>

Along the kynurenine pathway, picolinic acid is also described to exert potential effects on the immune system possessing both antimicrobial, antiviral and antifungal activity.<sup>68</sup> Picolinic acid enhances macrophage effector functions by favouring both the IFN- $\gamma$ -mediated increase of nitric oxide synthase gene expression<sup>204,205</sup> and the induction of the expression of macrophage inflammatory proteins (MIPs) 1 $\alpha$  and 1 $\beta$ .<sup>206</sup> The antimicrobial activity of picolinic acid seems to be caused by the ability of the molecule to chelate metal ions essential for bacteria, such as Fe<sup>2+</sup> ions.<sup>207,208</sup> Both, picolinic and quinolinic acids, can also enhance IFN- $\gamma$ -dependent inducible nitric oxide synthase (iNOS) expression involved in the immune response after gut microbiota exposure, thus modulating development of inflammatory responses.<sup>209,210</sup>

Indolic compounds may sustain gut immune responses by activating AhR.<sup>124</sup> In this latter context, AhR has been found in Th17 cells,<sup>211</sup> innate lymphoid cells,<sup>212,213</sup> macrophages,<sup>214</sup> DC,<sup>215,216</sup> and neutrophils.<sup>217</sup> IAld, for example, can activate AhR to regulate gut immune responses including interleukin-22 production and IEL recruitment.<sup>217,218</sup> *Lactobacillus Spp.* influenced interleukin IL-22 mucosal homeostasis via AhR activation by IAld, protecting mice against mucosal candidiasis.<sup>118</sup> Importantly, tryptophan metabolites via AhR may affect the differentiation of naïve CD4<sup>+</sup> T helper cells into either Treg or Th17 cells, which bears important consequences on the development of immune and inflammatory conditions.<sup>58</sup> In mice, activation of AhR by IAld and ILA produced after administration of *Lactobacillus reuteri* reprogrammed intraepithelial CD4<sup>+</sup> T helper cells into immunoregulatory T-cells (CD4<sup>+</sup>CD8<sup>+</sup> $\alpha\alpha$  double-positive IEL).<sup>218</sup> As part of an interkingdom bidirectional communication system, TLRs activation by microbial components, such as LPS and lipoteichoic acid, has been recognized as a key factor in the initialization of tryptophan metabolism along the kynurenine pathway.<sup>219</sup> Furthermore, stimulation of TLR-3 in monocytes may enhance kynurenine downstream production of both kynurenic and quinolinic acid.<sup>220</sup> Taken together, these observations suggest that development of research on the ability of tryptophan metabolites and their downstream

targets to influence the gut immune system may allow to establish new strategies to ameliorate the host health.

### The ENS

The ENS is constituted by a complex neuronal network that controls different gastrointestinal functions such as motility, secretion, mucosal transport, blood flow, and nutrient absorption and interacts with the gut immune and endocrine systems.<sup>40</sup> The ENS extends from the oesophagus to the colon and controls complex gastrointestinal responses, such as the peristalsis reflex, in rather autonomously with respect to the CNS and ANS.<sup>40</sup> However, the ENS is not totally autonomous and the complete regulation of the gastrointestinal functions originates from the integration of local reflexes, with reflexes involving sympathetic and vagal afferents from the gut to the CNS and vice versa.<sup>221</sup> The ENS is characterized by 3 major ganglionated plexuses, the submucosal, myenteric, and subserous plexus, separated by interconnecting fibre strands.<sup>36</sup> Enteric neurons are classified according to their morphology, neurochemical coding, projections to targets, and functional roles into 5 major types: intrinsic primary afferent neurons (IPAN), interneurons, excitatory and inhibitory motor neurons, and secretomotor neurons.<sup>36,40</sup> In the submucosal and myenteric plexus, IPANs possess large-cell bodies and bidirectional axons projecting to the mucosa to respond to chemical and mechanical stimuli giving rise to secretomotor, vasodilator, and motor reflexes.<sup>1,222</sup> This neuronal subtype is highly conserved in different animal species and can be identified by the presence of hyperpolarization-activated cation current and post-action potential and by the expression of different neurotransmitters such as substance P, acetylcholine, and calcitonin gene-related peptide and 5-HT.<sup>36,223,224</sup> In mammals, 5-HT released from ECs stimulates IPANs projecting to the mucosa, thus promoting activation of enteric reflexes.<sup>225</sup> Chemical and mechanical stimuli in the mucosa may be transduced by IPAN processes to descending and ascending interneurons and to excitatory and inhibitory motor neurons innervating the longitudinal and circular smooth muscle layers. 5-HT is also a neurotransmitter released from myenteric neurons that project in the descending pathway of the peristaltic reflex.<sup>225</sup> All together these observations point to 5-HT as an important mediator of gut motor function, although it is important to note that depletion of all endogenous 5-HT does not block peristalsis in the large intestine of vertebrates, nor inhibit transit.<sup>226</sup> Two major neurotransmitters, such as acetylcholine and tachykinins, are present in the excitatory motor neurons, whereas several neurotransmitters have been identified in inhibitory motor neurons, including nitric oxide (NO), VIP, and adenosine triphosphate (ATP)-like transmitters.<sup>72,227,228</sup> In several physiological and pathological conditions, the ENS undergoes plasticity, which is unmatched in any other section of the ANS.<sup>1,229,230</sup> The cross-talk among enteric neurons with several types of cells of the enteric microenvironment,

including enteric glial cells, smooth muscle cells, interstitial cells of Cajal, resident immune cells, and ECCs may, at least, explain such peculiar adaptation of the ENS.<sup>231-233</sup> In recent times, evidences are accruing to suggest that the gut microbiota, by either direct or indirect interaction with enteric neurons and glia, may influence the ENS structure and function.<sup>223,234</sup> In juvenile mice, induction of dysbiosis by chronic antibiotic treatment was followed by complex rearrangement within the ENS, including distortion of the enteric glial network alterations of both excitatory and inhibitory motor neurotransmission as well as upregulation of neurotrophic pathways such as brain-derived neurotrophic factor (BDNF) and its high-affinity receptor tropomyosin-related kinase B (TrkB) in sensory and motor myenteric neurons.<sup>223,234</sup> Interestingly, TLRs are located on myenteric and submucosal neurons as well as on enteric glial cells and may influence ENS integrity and function.<sup>235-237</sup> In mice, dysbiosis-induced neurochemical ENS derangement correlated with changes of TLR2 receptor distribution and slowing of the gastrointestinal transit, which was partially recovered by activation of TLR2 function.<sup>237</sup> Colonization of GF mice with a microbiota derived from conventionally raised mice also altered the ENS neurochemical coding, in particular the serotonergic pathways, and increased motor activity. These changes were dependent on the release of 5-HT from both ECs and enteric neurons and correlated with the proliferation of Nestin<sup>+</sup> enteric neuron progenitors in the adult gut.<sup>238</sup> In this latter regard, the role of 5-HT in adult ENS neurogenesis and neuroprotection is well-established ENS.<sup>102,238,239</sup> Tryptamine may indirectly stimulate gastrointestinal motility by inducing the release of 5-HT from ECs.<sup>240</sup> In mouse colonic epithelial cells, tryptamine was also shown to significantly affect ion secretion in vitro and to directly influence the transit of food particles and bacterial cells through the gut lumen.<sup>113</sup> Besides modulating ECs cells to shape ENS structure and function, the microbiota and its metabolites seem to directly influence IPANs.<sup>44,241</sup> In GF mice, a reduced excitability of myenteric IPANs was observed in vitro, which was restored after conventionalization with normal gut microbiota.<sup>242</sup> *Lactobacillus reuteri* increased the excitability and the number of action potentials per depolarizing pulse, decreased calcium and potassium channel opening, and reduced slow after-hyperpolarization in IPANs.<sup>44</sup> Specific bacterial strains may support the normal intestinal motor function as, in GF rats, derangement of the neuromuscular function and delay of intestinal transit were partially reversed after colonization with either *Lactobacillus acidophilus* or *Bifidobacterium bifidum*, while *Escherichia coli* and *Micrococcus luteus* delayed gut motility.<sup>243</sup>

### Microbiota-Gut-Brain Axis Dysfunction in IBS and IBD: Role of Tryptophan Metabolites

In recent times, data from metagenomics and metabolomics studies have greatly advanced our comprehension of the role of the gut microbiota and of its metabolites in the maintenance of

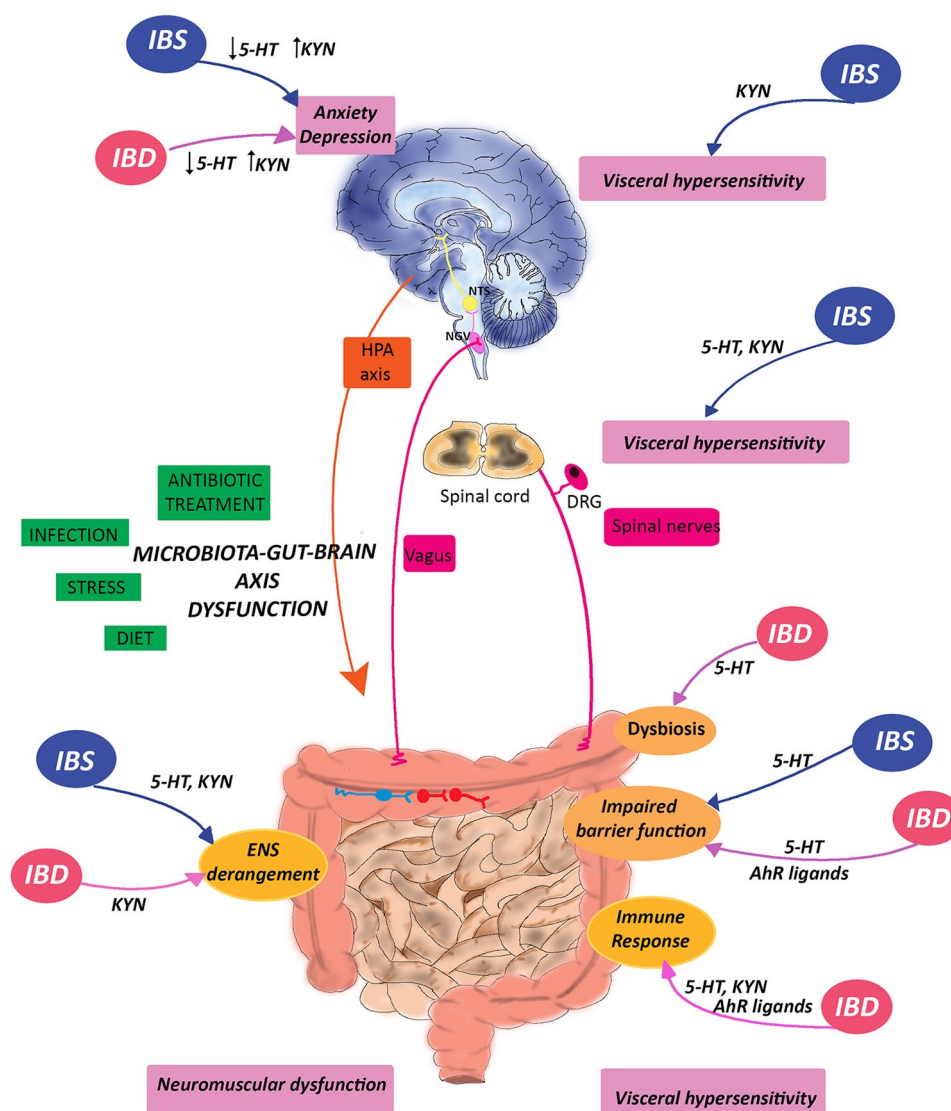
host health status. Unbalanced compositions of the microflora harbouring the human gut have been associated with numerous diseases, including gastrointestinal disorders.<sup>244-247</sup> Accordingly, data obtained from preclinical and clinical studies show that the pathogenesis of both IBS and IBD may, at least in part, depend on alterations of the saprophytic microflora, although a clear-cut demonstration of this correlation for both diseases has not yet been given.<sup>8,248</sup> Both in IBS and IBD, changes in the microbial community and in its metabolites participate in dysfunctions of the epithelial, immune, and neuronal gut compartments.<sup>1,3</sup> It is now assessed that such derangements may not only underlay gut symptoms but may also influence the gut-brain communication, predisposing to CNS disorders.<sup>12,249</sup> In this scenario, impaired tryptophan metabolism along the microbiota-gut-brain axis may potentially participate in the manifestation of both local and CNS symptoms associated with both diseases (Figure 3).

### *Irritable bowel syndrome*

Irritable bowel syndrome is the gut functional disorder with higher prevalence worldwide, showing a 2:1 ratio between females and males of prevalently less than 50 years of age.<sup>250</sup> Irritable bowel syndrome is a chronic or recurrent disorder, manifesting with abdominal pain and distension and altered bowel habits and disordered defecation, underlying either constipation (IBS-C) or diarrhoea (IBS-D) or both.<sup>11</sup> Symptoms develop as a consequence of dysmotility, visceral hyperalgesia, ANS dysfunctions, familiarity, psychosocial triggers, postinfectious events, and, as more recently suggested, microbiota-gut-brain dysfunctions, although the exact etiopathogenesis remains unknown.<sup>11,39,250</sup> Metagenomic studies suggest the existence of a correlation between changes in the gut microbiota composition and IBS development, with an increased ratio of *Firmicutes* to *Bacteroidetes* in all IBS patients<sup>11,251-253</sup> (Table 1). The hypothesis that changes in gut microbiota composition may correlate with the risk to develop IBS is inferred also from the evidence that a previous bacterial infection may predispose to IBS, the so-called post-infectious-IBS (PI-IBS).<sup>251</sup> Post-infectious-IBS patients have altered sensory and motor intestinal responses, which depend, at least in part, on a subclinical low-grade immune activation, in the absence of overt signs of gut inflammation. Interestingly, colonic biopsies from IBS patients show enhanced number of immunocytes and receptors for bacterial metabolites, such as TLRs.<sup>254-256</sup> There are indications that impairment of tryptophan metabolism may correlate to IBS pathogenesis.<sup>10,219</sup> Alteration of 5-HT homeostasis has been described in IBS patients, which may relate to dysmotility. In IBS patients, 5-HT mucosal content is lower than in healthy controls and is associated with lower expression levels of TPH1 and serotonin reuptake transporter.<sup>257-259</sup> Changes in 5-HT levels may, however, differ according to the IBS subtype, as colonic levels are prevalently reduced in IBS-C and increased in IBS-D.<sup>260</sup>

Accordingly, 5-HT levels may be upregulated in PI-IBS development. In this condition, inflammation-induced upregulation of 5-HT signalling persists after the inflammation has ceased, and microbial activation of ECs signalling may favour symptom persistence in PI-IBS.<sup>261,262</sup> Serotonergic receptors of the 5HT<sub>3</sub> type are altered in IBS patients and administration of 5-HT<sub>3</sub> receptor antagonists to IBS patients slowed colonic transit, enhanced small intestinal absorption, and reduced visceral pain by activation of gut-brain pathways.<sup>263,264</sup> A recent meta-analysis review of the literature has demonstrated that different 5-HT<sub>3</sub> receptor antagonists may represent valid therapeutic tools to treat IBS and IBS-C with few associated adverse effects.<sup>265</sup> A detailed description of 5-HT involvement IBS-associated gut motor function derangements is, however, beyond the scope of this review and has been elegantly detailed elsewhere.<sup>98</sup>

*Visceral pain as a microbiota-gut-brain axis dysfunction in IBS.* In IBS patients, alterations of the microbial flora may underlie visceral hyperalgesia, defined as a poorly localized and diffuse chronic abdominal pain<sup>221,266</sup> (Table 1). In particular, changes in the amounts of specific phyla, such as Proteobacteria, were correlated with the scores of visceral pain in IBS patients.<sup>252,267</sup> Alterations in the microbial saprophytic flora may favour development of visceral pain during the whole lifespan, for example, the occurrence of dysbiosis in the early periods of life favours development of visceral pain during adult age.<sup>268</sup> This observation is all the more important considering that repeated courses of antibiotics during childhood may be associated with an increased risk of visceral pain during adulthood.<sup>269</sup> Preclinical studies have demonstrated that both IPANs and extrinsic sensory afferents modulating visceral pain are influenced by the microflora.<sup>243,270</sup> From a translational viewpoint, numerous studies carried out on GF and dysbiotic murine models have demonstrated the potential beneficial effects of microbiota manipulation via probiotic or antibiotic treatment on visceral pain perception.<sup>271-274</sup> Several hints suggest that tryptophan metabolites, such as 5-HT and kynurenines, are involved in the modulation of both local and ascending neuronal pathways transmitting visceral pain perception along the microbiota-gut-brain axis. Release of 5-HT from colonic mucosa correlated with the severity of abdominal pain/discomfort in patients with IBS.<sup>263</sup> It is unlikely that variations of 5-HT release from ECs may influence visceral pain perception by exerting a direct effect on the CNS, as 5-HT cannot cross the BBB.<sup>275</sup> Most probably, 5-HT is locally involved in visceral hyperalgesia by influencing vagal afferents along the brain-gut axis<sup>98,276</sup> as well as inflammatory responses in the gut.<sup>277</sup> Colonic mucosal supernatants of IBS patients increased the firing rate of rat mesenteric sensory neurons in vitro and this excitatory effect was inhibited by the 5-HT<sub>3</sub> receptor antagonist, granisetron.<sup>263</sup> Other investigations have demonstrated that both peripheral 5-HT<sub>4</sub> and 5HT<sub>3</sub> receptors are involved in the modulation of visceral pain associated with IBS, suggesting that modulation



**Figure 3.** Potential role of tryptophan metabolites in the modulation of symptoms associated with IBS and IBD. Environmental changes, stress, diet, previous infection, and dysbiosis may alter the homeostasis of the gut-brain axis underlying development of motor dysfunction, visceral pain, and CNS disorders, such as anxiety and depression in both IBS and IBD patients. Tryptophan metabolites by influencing the epithelial barrier, the immune function, and the ENS may peripherally influence symptom development, as detailed in the text. Furthermore, 5-HT and kynurenine may participate in behavioural disorders associated with both diseases. CNS indicates central nervous system; DRG, dorsal root ganglion; ENS, enteric nervous system; HPA, hypothalamic-pituitary axis; IBD, inflammatory bowel disease; IBS: irritable bowel syndrome; KYN, kynurenine; NGV, nodose vagal ganglion; NTS, nucleus of the solitary tract.

Source: adapted from Baj et al.<sup>1</sup>

of 5-HT receptors may represent a useful therapeutic strategy.<sup>98,102,264</sup> As the microbiota participates in pain signalling from the gut,<sup>4</sup> it is reasonable that 5-HT producing microbes, such as *Streptococcus*, *Escherichia*, and *Enterococcus*, may influence visceral pain perception<sup>102</sup>; however, further research is necessary to confirm this issue.<sup>278</sup> Although the majority of studies regarding the involvement of gut tryptophan metabolites in the modulation of visceral pain perception have focused on 5-HT, kynurenes may also have a role, representing a possible novel signalling pathway to target visceral hyperalgesia in IBS. Indeed, kynurenine levels increase in the serum of IBS patients<sup>279</sup> and the peripheral IDO1 activity positively correlates with IBS severity.<sup>280</sup> In the kynurenine

pathway, kynurenic acid, as a modulator of glutamatergic NMDA receptor pathways, may have a role in development of IBS-associated visceral pain. Along the gastrointestinal tract, glutamate, via NMDA receptors, may act as an endogenous modulator of mechanosensitive pathways, transducing sensory stimuli deriving from pelvic and splanchnic afferents in response to neuroinflammation and hyperalgesia.<sup>281-285</sup> Activation of NMDA receptors in the CNS was also involved in the visceromotor responses to noxious colorectal distension, and this effect was blocked by the antagonist at the glycine site associated with NMDA receptor, 7-chloro-kynurenic acid, a derivative of kynurenic acid.<sup>286,287</sup> Interestingly, in preclinical models of IBS, dysbiosis was correlated with changes in

**Table 1.** Examples of microbiota-gut-brain axis abnormalities associated with IBS.

MODEL	FINDINGS	REF.
Gut microbiota analysis in patients with IBS	Increased ratio of <i>Firmicutes</i> to <i>Bacteroidetes</i>	Holtmann et al <sup>11</sup> and Jeffery et al <sup>252</sup>
	Lower diversity in the microbiota composition of IBS patients	Jeffery et al <sup>252</sup>
	Microbial signature at the specie levels is not uniform and depends on the subgroup of IBS patients considered	Krogus-Kurikka et al <sup>253</sup> and Bercik et al <sup>254</sup>
Expression levels of microbial sensing receptors (TLRs) in IBD patients	Significantly increased levels of TLR2 receptor in colonic biopsies from IBS-D patients	Belmonte et al <sup>255</sup>
	Significantly increased levels of TLR4 receptor in colonic biopsies from IBS-D and IBS-C patients	Belmonte et al <sup>255</sup>
	Increased expression of TLR2 and TLR4 receptors in epithelial cells of the colonic mucosa significantly correlating with IBS-M symptoms	Koçak et al <sup>256</sup>
Clinical studies of visceral hyperalgesia	Increased amount of faecal Proteobacteria positively correlating with visceral pain scores in adult IBS patients	Krogus-Kurikka et al <sup>253</sup>
	Increased amount of the genus <i>Alistipes</i> ( <i>Bacteroidetes</i> ) associated with recurrent abdominal pain in paediatric IBS	Desbonnet et al <sup>268</sup>
	Repeated courses of antibiotics during the first and second year of life increase the risk of visceral pain in an adolescent female population	Perez-Burgos et al <sup>270</sup>
Preclinical studies of visceral hyperalgesia	Early postnatal exposure to antibiotics predisposes to development of visceral hypersensitivity in adult rats.	Uusijärvi et al <sup>269</sup>
	In GF mice, myenteric IPANs display reduced excitability, which can be restored by microbiota re-colonization	Garrett <sup>244</sup>
	Administration of live <i>Lactobacillus reuteri</i> (DSM 17938) reduces spinal nerve firing from the mouse jejunum after gut distension or capsaicin treatment	McKernan et al <sup>271</sup>
	Probiotic treatment ( <i>Bifidobacterium infantis</i> 35624) for 14 days reduces visceral pain perception to CRD in adult rats	Aguilera et al <sup>272</sup>
	2-week antibiotic treatment in adult mice modulates neuroimmune sensory pathways and reduces visceral pain responses related to intraperitoneal or intracolonic chemical stimulation	Verdú et al <sup>273</sup>
	Administration of <i>Lactobacillus paracasei</i> NCC2461 to dysbiotic adult mice reduces visceral hypersensitivity to CRD	Crouzet et al <sup>274</sup>
	GF rats transplanted with faecal microbiota from IBS-C patients experiencing hypersensitivity to CRD display visceral sensitivity. This effect is not reproduced in rats receiving faecal transplantation from healthy volunteers	Donovan and Tecott <sup>275</sup>
Clinical and preclinical studies correlating the microbiota with stress-related psychiatric disorders	Significant correlation between microbial signature and clinically relevant depression in IBS patients	Krogus-Kurikka et al <sup>253</sup> and Bercik et al <sup>254</sup>
	Faecal microbiota transplantation from depressed patients to GF mice results in the development of a depressive-like phenotype	Jiang et al <sup>296</sup>
	Faecal transplantation from depressed patients to antibiotic-treated rats favours the development of anhedonia and anxiety-like behaviour	Sudo et al <sup>299</sup>
	GF mice exhibit an exaggerated HPA stress response concomitant with alterations in the expression of neuroplasticity modulators (BDNF and NMDA receptors) in hippocampus and cortex. HPA axis response to stress in GF mice are reversed by re-colonization with <i>Bifidobacterium infantis</i> at an early age	Gareau et al <sup>300</sup>

(Continued)

Table 1. (Continued)

MODEL	FINDINGS	REF.
	In mouse pups, maternal separation, representing a model of early life stress and trauma, enhances HPA axis activity and colonic epithelial barrier dysfunction correlating to impaired <i>Lactobacillus</i> colonization. Administration of probiotics of the <i>Lactobacillus spp.</i> restores colonic flora and reduces HPA axis activity	Lafuse et al <sup>301</sup>
	Prolonged exposure to stress causes ultrastructural alterations of the intestinal barrier favouring systemic translocation of different bacteria strains, such as <i>Lactobacillus spp.</i> and activation of an immune response	Lyte et al <sup>302</sup>
	Stress-related neurotransmitters, such as catecholamines, favour bacteria growth (ie, nonpathogenic <i>Escherichia coli</i> and pathogenic <i>Escherichia coli</i> O157: H7 strains)	Marin et al <sup>303</sup>

Abbreviations: BDNF, brain-derived neurotrophic factor; CRD, colorectal distention; GF, germ-free; HPA, hypothalamus-pituitary axis; IBS, irritable bowel syndrome; IBS-C, IBS with constipation; IBS-D, IBS with diarrhoea; IBS-M, mixed constipation and diarrhoea IBS; IPAN, intrinsic primary afferent neurons; NMDA, N-methyl-D-aspartate (ionotropic glutamate receptor); TLR, Toll-like receptor; WT, wild type.

NMDA receptor expression, both in the gut and in the CNS.<sup>288</sup> Modulation of glutamatergic transmission along the gut-brain axis may participate to adaptation of afferent neurons and CNS pain processing, leading to chronic visceral hypersensitivity in IBS patients.<sup>221</sup> In particular, NMDA receptors located on complex neuronal networks in the spinal cord may promote the amplification of nociceptive signals and the 'wind-up' of central responses to nociceptive stimuli.<sup>221,266</sup> Several studies suggest that kynurenic acid and its synthetic analogues may offer novel therapeutic options for the treatment of neuropathic pain syndromes devoid of significant adverse effects.<sup>289-291</sup>

*Tryptophan metabolites and IBS-associated anxiety and depression.* IBS patients may frequently experience high levels of anxiety and depression with respect to healthy subjects, regardless of the IBS subtype.<sup>292</sup> Both psychiatric disorders are closely related to stress, an important pathogenic factor for IBS.<sup>249,293</sup> Stress is considered a dynamic process in which physical and/or mental homeostasis may be altered by both exogenous and endogenous stressors.<sup>294</sup> Stress-induced disorders developing in IBS patients may relate to dysbiosis (Table 1).<sup>251,252</sup> Association of major depressive disorders with changes in gut microbiota have given different outcomes concerning the phylum Bacteroidetes, as some reports show a reduction<sup>295</sup> or an enhancement.<sup>296,297</sup> Studies carried out in GF or dysbiotic murine models suggest that the interplay between the gut microbiota, the HPA axis, and neuroimmune pathways is fundamental in driving the host response to stress stimuli.<sup>295,298-302</sup> From a metabolomic stand point, changes in microbiota composition during chronic stress is associated with the diversion of tryptophan metabolism from the 5-HT to the kynurenine pathway.<sup>9</sup> Administration of the probiotic *Lactobacillus reuteri* in mice subjected to stress stimuli attenuated the unbalanced tryptophan transformation, reinforcing the concept that the microbiota may influence metabolism and resilience during stress.<sup>303</sup> In GF mice, faecal transplantation induced a depressive mood which was associated with an enhancement of the kynurenine/tryptophan ratio.<sup>35</sup> Both *in vitro* and *in vivo*

studies showed that IDO1 activation in the presence of IFN $\gamma$  favours the conversion of tryptophan into kynurenine, promoting the development of depressive symptoms.<sup>304</sup> Interestingly, anxiety-like behaviours developed in mice receiving gut microbiota from depressed patients in parallel with increased blood kynurenine levels and kynurenine/tryptophan ratio.<sup>297</sup> In rats, supplementation of *Bifidobacterium infantis* ameliorated the depressive behaviour induced by a forced-swim test and normalized tryptophan metabolism.<sup>91</sup> Manifestation of depressive symptoms in IBS patients was correlated with altered tryptophan metabolism.<sup>196</sup> In IBS patients, IDO1 activity is enhanced and positively linked with the severity of the disease, while kynurenic acid levels and the ratio between kynurenic acid/kynurenine is reduced.<sup>305</sup> A significant correlation was observed between the decrease of kynurenic acid and 5-HT in duodenal mucosal biopsy specimens and the psychological index state of IBS patients.<sup>306</sup> Kynurenine involvement in stress-related disorders may depend on the ability of this molecular pathway to influence NMDA receptors located in CNS regions involved in the development of depression, such as hippocampus, amygdala, and cingulate cortex.<sup>7,9,307</sup> Both preclinical and clinical studies indicate that NMDA receptor blockade may have anxiolytic and antidepressant effects.<sup>309-311</sup> In mice, the prodrug, 4-chloro-kynurenic acid, induced a rapid onset of antidepressant effects, displaying a better safety profile than other glutamatergic antagonists already used in clinical settings, such as ketamine, holding promises for use in humans.<sup>310,311</sup>

### Inflammatory bowel disorders

Inflammatory bowel disease, including Crohn disease (CD), with the inflammatory response developing along the whole gastrointestinal tract, and ulcerative colitis (UC), with the inflammation constrained to the rectum and colon, displays an increasing incidence worldwide.<sup>312,313</sup> The etiopathogenesis remains to be clear-cut defined although there is consensus to suggest that on several factors including host genetics, immune responses, the gut microbiota, and environmental stimuli

**Table 2.** Examples of microbiota-gut-brain abnormalities associated with IBD.

MODEL	FINDINGS	REF.
Gut microbiota analysis in patients with IBD	Reduced amounts of faecal Firmicutes (especially <i>Faecalibacterium prausnitzii</i> in adult CD patients) Decrease in the diversity of Firmicutes constituent species in IBD patients	Barnich et al <sup>315</sup>
	Enhanced levels of Proteobacteria ( <i>Escherichia coli</i> )	Frank et al <sup>316</sup>
	Reduction of <i>Clostridium clusters XIVa and IV (Faecalibacterium prausnitzii)</i> correlated with recurrence of CD after surgery	Walker et al <sup>317</sup> and Hoshi et al <sup>318</sup>
Development of spontaneous colitis in transgenic mouse models	Adoptive T-cell transfer to SCID or Rag <sup>-/-</sup> recipient mice lacking adaptive immunity induces an IL-23-mediated spontaneous colitis dependent on the microbiota composition	Ni et al <sup>8</sup>
	In IL10 <sup>-/-</sup> mice spontaneous colitis develops only in the presence of a healthy microbiota and is determined by unopposed Th17 cells	Gasche et al <sup>319</sup>
GWAS studies of microbial sensing receptors (TLRs) gene expression in IBD patients	Positive association between TLR4 gene variants and development of both CD and UC	De Jager et al. <sup>320</sup>
	Positive association between nonsynonymous variants in TLR1, -2 and -6 genes with extensive colonic disease in both UC and CD	Pierik et al. <sup>321</sup>
Severity of DSS-induced colitis in mice	TLR2 <sup>-/-</sup> mice develop a more severe colitis and epithelial damage with respect to WT	Brun et al <sup>237</sup>
	In mice lacking the TLR downstream signalling, MyD88, the degree of DSS-induced colitis is severe with erosion of the epithelium and ulceration	Ni et al <sup>8</sup>
	Conditional mice expressing MyD88 in myeloid lineages display recruitment of stromal and myeloid cells to colonic crypts and epithelial repair after DSS treatment	Malvin et al <sup>322</sup>
	Inflammation injury in TLR2 <sup>-/-</sup> and MyD88 <sup>-/-</sup> mice induces early tight-junction-associated barrier disruption and correlates with anti-apoptotic failure of the epithelial barrier; <i>In vitro</i> and <i>ex vivo</i> TLR2 stimulation by the synthetic ligand Pam3Cys-Sk4 protects tight-junction-associated barrier assembly against inflammation-induced injury, via MyD88; Oral treatment with Pam3Cys-Sk4 suppresses mucosal inflammation and apoptosis, restoring the tight-junction-associated epithelium integrity	Cario et al <sup>147</sup>
	In TLR2 <sup>-/-</sup> mice, epithelial injury is reduced after improvement of ENS alterations by GDNF administration	Brun et al <sup>237</sup>
Anxiety-like behaviours and cognitive deficits after DSS-induced colitis in mice	Impaired recognition memory and anxiety-like behaviour decrease in <i>Lactobacillus spp.</i> and SBF during active inflammation. Reduction of behavioural anomalies after supplementation of <i>Lactobacillus rhamnosus</i> R0011 and <i>Lactobacillus helveticus</i> R0052	Bercik et al <sup>356</sup>
	Normalization of anxiety-like behaviours after administration of <i>Bifidobacterium longum</i> NCC3001 requires vagal integrity; <i>Bifidobacterium longum</i> NCC3001 decreases excitability of ileal myenteric plexus neurons, suggesting that the probiotic may promote the activation of an ENS-CNS cross-talk via the vagus nerve	Bercik et al <sup>357</sup>

Abbreviations: CD, Crohn disease; CNS, central nervous system; DSS, dextran sodium sulphate; ENS, enteric nervous system; GDNF, glial-derived neurotrophic factor; GWAS, Genome-Wide Association Study; IBD, inflammatory bowel disease; SBF, segmented filamentous bacteria; SCID, severe combined immunodeficiency; TLR, Toll-like receptor; UC, ulcerative colitis; WT, wild type.

contribute to the disease.<sup>8,313</sup> The pathophysiological relevance of the gut microbiota in IBD has been proposed after different preclinical and clinical observations, showing that IBD patients often develop dysbiosis<sup>8,314-318</sup> and that some antibiotics may be useful to prevent or treat inflammation both in humans and in animal models<sup>8,313</sup> (Table 2). Inflammatory bowel disease patients manifest signs of alteration of all the mechanism

involved in the oral tolerance process,<sup>186</sup> with low levels of IL-10 in the intestinal mucosa leading to the maturation of DC and the stimulation of Th1 pro-inflammatory response.<sup>319</sup> Interestingly, in transgenic mice lacking IL-10, development of a spontaneous inflammation is strictly correlated to the composition of microbial flora.<sup>318</sup> Genome-wide associated studies evidenced a possible pathogenetic role for TLR gene variants

in IBD,<sup>320,321</sup> while preclinical models of IBD have shown positive correlations between the severity of the disease and alterations in TLR signalling pathways.<sup>8,147,237,322</sup> IBD is also associated with alterations in sensory, motor, and secretory gut functions, suggesting the involvement of the ENS.<sup>228-230,323</sup> Such neuroplastic changes may result from the interaction between enteric neurons and glia with immunocytes but also with gut microbes.<sup>230,324</sup> The discovery of possible modulators of this microbiota-immune-neuronal axis is particularly important to prevent manifestations of more overt inflammatory conditions. Tryptophan and its metabolites may have a role in this context. In IBD patients, tryptophan levels are lower than in healthy controls, and this reduction is particularly evident in CD patients as compared to UC patients, and correlates with the gravity of the disease.<sup>325-327</sup> The reduction of tryptophan serum levels during active IBD is associated with an increased aminoacid metabolism towards the kynurenine arm, leading to elevated quinolinic acid serum levels.<sup>325,327</sup> From a translational view point, mice treated with dextran sulphate sodium (DSS) to induce colitis were more prone to develop inflammation after administration of a tryptophan-free diet.<sup>328</sup> Several factors such as cytokines, cortisol, and the perturbed microbiota may contribute to IDO activation and to the enhancement of kynurenine levels during gut inflammation.<sup>329-331</sup> In IDO1<sup>-/-</sup> mice, trinitro-benzen-sulfonic acid (TNBS) induced a more severe colitis than in the corresponding wild-type animals, possibly by increasing the release of pro-inflammatory cytokines and decreasing the level of CD4<sup>+</sup> Foxp3<sup>+</sup> regulatory T-cells in the colon.<sup>332</sup> Increased serum and endoscopic levels of kynurenine acid were also found in IBD patients.<sup>333,334</sup> Upregulation of IDO1 is a hallmark of many other diseases, including metabolic disorders such as obesity and insulin resistance, all characterized by a low-grade, Th1-sustained inflammation. In this context, the kynurenine pathway seems to have an important immunomodulatory role, serving as a modulator to reduce the immune system activation by reducing Th1 responses and enhancing Th2-mediated processes.<sup>335,336</sup> However, these beneficial effects may be blunted by the detrimental consequences of the increase in neurotoxic metabolites such as quinolinic acid.<sup>335</sup>

In the gut, the modulation of the kynurenine pathway in favour of kynurenine acid synthesis may be neuroprotective to compensate for inflammation-induced increased NMDA receptor activity in the neuromuscular compartment.<sup>281</sup> Enteric NMDA receptor pathways may sustain development of neuro-inflammatory responses by promoting the activation of oxidative and nitrosative stress pathways.<sup>337</sup> In this context, both preclinical and human studies have proposed kynurenine acid and its derivative, as potential therapeutic tools for IBD management.<sup>337</sup> In the dog and rat colon, administration of kynurenine acid during the acute phase of an experimentally induced inflammation reduced motility index, NO, and ROS levels and peroxynitrite

production.<sup>201,338</sup> The adaptation of enteric neurons during inflammation depends on the intensity of the injury as well as from its acute vs chronic form.<sup>323,324</sup> Enduring and severe inflammation is associated with enhanced production of different pro-inflammatory cytokines, such as IL-1 $\beta$ , IL-6, and TNF $\alpha$ , and with increased NMDA-mediated NO transmission, with consequent alteration of the motor function.<sup>337,339</sup> In rats treated with TNBS to induce colitis, administration of kynurenine acid and SZR-72, a centrally-acting kynurenine acid analogue, reduced nitrosative stress, IL-6, and TNF $\alpha$  production and ameliorated the motility patterns suggesting the involvement of both peripheral and central NMDA receptor pathways.<sup>340</sup> Overall, these data indicate that modulation of the glycine site associated with NMDA receptors with kynurenine acid may represent a promising therapeutic approach to treat neuromuscular dysfunctions associated with IBD.

Recent studies suggest that the AhR has a protective role during intestinal inflammation.<sup>58</sup> In a humanized murine model whereby human CD4<sup>+</sup> T-cells drive colitis on exposure to TNBS, activation of AhR ameliorated colitis-induced Treg cells, thus promoting oral immune tolerance.<sup>341</sup> Downregulation of AhR has been demonstrated both in animal models of colitis and in intestinal tissue of IBD patients.<sup>342,343</sup> In this latter study, pharmacological manipulation of AhR on mononuclear cells isolated from the intestinal mucosa reduced the expression levels of the pro-inflammatory cytokine, IFN $\gamma$ , and upregulated IL-22.<sup>342</sup> Changes in serum and faecal levels of several AhR ligands were also observed both in preclinical models of colitis and in IBD patients.<sup>10,58</sup> In a caspase recruitment domain-containing protein 9 (Card9) knockout mouse model of DSS-induced colitis, the dysbiotic microbiota could not catalyse tryptophan into IAA, leading to reduced IL-22 and higher susceptibility to inflammation.<sup>344</sup> Selective depletion of IPA was demonstrated by means of a metabolomic profiling approach in circulating serum from patients with active colitis with respect to healthy subjects.<sup>345</sup> Isolated intestinal epithelial cells exposed to indole metabolites overexpressed the IL-10 receptor ligand-binding subunit (IL-10R1), which attenuates excessive production of pro-inflammatory mediators in ECs during inflammation.<sup>345</sup> Moreover, oral administration of both indole and IPA significantly ameliorated colitis in chemically induced inflammation in mouse small and large intestine.<sup>345,346</sup> Strains of *Lactobacillus* with AhR activating ability reduced the severity of DSS-induced colitis.<sup>344,347</sup> Indole acrylic acid produced by *Peptostreptococcus spp.* reduced the susceptibility to colitis by improving goblet cell differentiation and reducing inflammatory signals.<sup>139</sup> The observation that a bacterium with mucin- and tryptophan-metabolizing abilities may ameliorate epithelial integrity led the A32wuthors to identify a reduced abundance of phenyllactate gene cluster in ulcerative colitis by metagenomic analysis.



The relevance of 5-HT in the pathogenesis of IBD is less clear-cut defined. 5-HT serum levels were found to vary, although with different results, either enhancing or decreasing in dependence of whether the amine levels were measured in CD or UC patients, respectively.<sup>260</sup> In TNBS and DSS experimental models of colitis, enhancement of mucosal 5-HT content was observed.<sup>348,349</sup> In TPH1<sup>-/-</sup> mice, the severity of chemically induced colitis was reduced with respect to the corresponding wild type, and restoration of 5-HT levels by administration of a 5-HT precursor intensified the severity of colitis.<sup>350</sup> In addition, in transgenic mouse models, the severity of spontaneous colitis associated with IL-10 deficiency increased when coupled with 5-HT reuptake transporter (SERT) knockout inducing increased 5-HT levels.<sup>351,352</sup> Consistent with the existence of a bidirectional communication system between the microbiota and the host, in a recent study microbial transfer from *Tpb1*<sup>-/-</sup> to either *Tpb1*<sup>+/-</sup> littermates, displaying different microbial composition, or to GF mice had protective effects on DSS-induced colitis injury, suggesting that gut-derived 5-HT has a role in shaping gut microbiota composition in relation to predisposition to colitis.<sup>353</sup>

Overall, these observations suggest that tryptophan metabolism underlays IBD pathogenesis. Alterations in the saprophytic microflora may contribute to disease development, either by influencing AhR ligand levels or by modulating the host IDO and TPH1 activity. In this view, manipulation of tryptophan metabolism either via conventional pharmacological approaches or by administration of pre- and probiotics targeting tryptophan metabolite-producing bacteria may represent promising novel therapeutic approaches in IBD patients.

*Kynurenine pathways and IBD-associated psychiatric disturbances.* In IBD patients, stress-related disorders, such as major depression and generalized anxiety, are common symptoms which influence the outcome of disease treatment and may be correlated with changes in the gut microbiota composition and function.<sup>12,354</sup> Anxiety-like behaviours have been also demonstrated in murine models of colitis<sup>355,356</sup> (Table 2). In mice treated with the non-invasive parasite, *Trichuris muris* to induce an experimental colitis, psychological disturbances were associated with enhancement of circulating kynurenine levels and enhanced kynurenine/tryptophan ratio.<sup>357</sup> Interestingly, administration of current therapeutic anti-inflammatory agents for IBD, such as etanercept and budesonide, to mice reduced kynurenine levels and normalized behaviour.<sup>357</sup> An unbalanced kynurenine/tryptophan ratio and diversions of tryptophan metabolism from 5-HT synthesis to kynurenine and its downstream metabolites may underlie psychological syndromes in IBD.<sup>1,9</sup> Indeed, changes of 5-HT homeostasis may underlay mood disorders in IBD patients.<sup>49,102</sup> During gut inflammation, kynurenine translocation in the brain, through the BBB, is favoured by the enhanced circulating levels. In the CNS, the amine is transformed into its metabolites, principally kynurenic

acid and quinolinic acid.<sup>102</sup> Quinolinic, as a neurotoxic NMDA agonist, has been suggested to participate to depression development.<sup>49</sup> Data from clinical investigations suggest that kynurenic acid/quinolinic acid ratio may represent an index of neuroprotection, and a reduced ratio may be indicative of inflammation-induced depressive disorders.<sup>358</sup> Overall, these observations suggest that modulation of tryptophan metabolism may represent a unifying mechanism linking inflammation-induced depression and dysbiosis along the microbiota-gut-brain axis.<sup>49</sup>

## Conclusions

The gut microbiota has an elevated capability to adapt to changes in the host life-style (determined by diet, drugs, social, ethnic and environmental factors) and our behaviour may deeply influence this symbiotic organ. Conversely, microbes inhabiting our body may interfere with the host gut and brain functions by releasing bioactive molecules, via humoral, endocrine, immune, and neuronal pathways. In the present dissertation, we have shown that tryptophan metabolites have a central role in the regulation of this bidirectional microbiota-gut-brain axis in both physiological and pathological conditions. In particular, an increasing number of studies suggest that 5-HT, kynurenine, and AhR ligand pathways are involved in the pathogenesis of 2 major gastrointestinal diseases such as IBS and IBD, both characterized by psychiatric disorders. As tryptophan metabolites are directly or indirectly controlled by the gut microbiota, this opens the intriguing perspective that modulation of tryptophan metabolism, either by conventional pharmacological tools or by influencing the microbiota composition with pre- or probiotics, may represent a useful therapeutic approach. However, caution must be taken when considering the relationship between tryptophan metabolites with the host health, as the majority of studies in this field, at the moment, have been carried out on preclinical mouse models. Therefore, a more comprehensive understanding of the pathophysiological dynamics involving tryptophan metabolism and their functional implications in IBS and IBD involves the conduction of large-scale, highly controlled clinical studies. Furthermore, a promising strategy will be to combine different methodological approaches of metabolomics, metagenomics, metatranscriptomics, and proteomics to identify bacteria and bacterial genes involved in the modulation of tryptophan metabolite signalling and to verify their potential efficacy as adjuvant in the therapy of IBD and IBS and the related gut-brain axis disorders.

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## Author Contributions

AB, DB and MB wrote and discussed the manuscript; CG and AB conceived, wrote, discussed and revised the manuscript

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## REFERENCES

- Baj A, Moro E, Bistoletti M, Orlandi V, Crema F, Giaroni C. Glutamatergic signaling along the microbiota-gut-brain axis. *Int J Mol Sci.* 2019;20:1482. doi:10.3390/ijms20061482.
- Rhee SH, Pothoulakis C, Mayer EA. Principles and clinical implications of the brain-gut-enteric microbiota axis. *Nat Rev Gastroenterol Hepatol.* 2009;6:306-314. doi:10.1038/nrgastro.2009.35.
- Sekirov I, Russell SL, Cactano M, Antunes L, Finlay BB. Gut microbiota in health and disease. *Physiol Rev.* 2010;90:859-904. doi:10.1152/physrev.00045.2009.
- Cryan JF, O'Riordan KJ, Cowan CSM, et al. The microbiota-gut-brain axis. *Physiol Rev.* 2019;99:1877-2013. doi:10.1152/physrev.00018.2018.
- Mittal R, Debs LH, Patel AP, et al. Neurotransmitters: the critical modulators regulating gut-brain axis. *J Cell Physiol.* 2017;232:2359-2372. doi:10.1002/jcp.25518.
- Kelly JR, Minuto C, Cryan JF, Clarke G, Dinan TG. Cross talk: the microbiota and neurodevelopmental disorders. *Front Neurosci.* 2017;11:490. doi:10.3389/fnins.2017.00490.
- Bastiaansen TFS, Cowan CSM, Claesson MJ, Dinan TG, Cryan JF. Making sense of . . . the microbiome in the psychiatry. *Int J Neuropsychopharmacol.* 2019;22:37-52. doi:10.1093/ijnp/pyy067.
- Ni J, Wu GD, Albenberg L, Tomov VT. Gut microbiota and IBD: causation or correlation? *Nat Rev Gastroenterol Hepatol.* 2017;14:573-584. doi:10.1038/nrgastro.2017.88.
- Kennedy PJ, Cryan JF, Dinan TG, Clarke G. Kynurenine pathway metabolism and the microbiota-gut-brain axis. *Neuropharmacology.* 2017;112:399-412. doi:10.1016/j.neuropharm.2016.07.002.
- Agus A, Planchais J, Sokol H. Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host Microbe.* 2018;23:716-724. doi:10.1016/j.chom.2018.05.003.
- Holtmann GJ, Ford AC, Talley NJ. Pathophysiology of irritable bowel syndrome. *Lancet Gastroenterol Hepatol.* 2016;1:133-146. doi:10.1016/S2468-1253(16)30023-1.
- Mikocka-Walus A, Knowles SR, Keefer L, Graff L. Controversies revisited: a systematic review of the comorbidity of depression and anxiety with inflammatory bowel diseases. *Inflamm Bowel Dis.* 2015;22:752-762. doi:10.1097/MIB.0000000000000620.
- Gordon JI. Honor thy gut symbionts redux. *Science.* 2012;336:1251-1253. doi:10.1126/science.1224686.
- Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut microbiota in the pathogenesis of inflammatory bowel disease. *Clin J Gastroenterol.* 2018;11:1-10. doi:10.1007/s12328-017-0813-5.
- Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. *Sci Transl Med.* 2014;6:237ra65. doi:10.1126/scitranslmed.3008599.
- Fernández L, Langa S, Martín V, et al. The human milk microbiota: origin and potential roles in health and disease. *Pharmacol Res.* 2013;69:1-10. doi:10.1016/j.phrs.2012.09.001.
- Lloyd-Price J, Abu-Ali G, Huttenhower C. The healthy human microbiome. *Genome Med.* 2016;8:51. doi:10.1186/s13073-016-0307-y.
- Sharon G, Sampson TR, Geschwind DH, Mazmanian SK. The central nervous system and the gut microbiome. *Cell.* 2016;167:915-932. doi:10.1016/j.cell.2016.10.027.
- Methé BA, Nelson KE, Pop M, et al. A framework for human microbiome research. *Nature.* 2012;486:215-221. doi:10.1038/nature11209.
- Rodríguez JM, Murphy K, Stanton C, et al. The composition of the gut microbiota throughout life, with an emphasis on early life. *Microb Ecol Health Dis.* 2015;26:26050. doi:10.3402/mehd.v26.26050.
- Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc Natl Acad Sci U S A.* 2011;108:4554-4561. doi:10.1073/pnas.1000087107.
- Yatsunen T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. *Nature.* 2012;486:222-227. doi:10.1038/nature11053.
- Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Reddy DN. Role of the normal gut microbiota. *World J Gastroenterol.* 2015;21:8836-8847. doi:10.3748/wjg.v21.i29.8787.
- López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell.* 2013;153:1194. doi:10.1016/j.cell.2013.05.039.
- Biagi E, Franceschi C, Rampelli S, et al. Gut microbiota and extreme longevity. *Curr Biol.* 2016;26:1480-1485. doi:10.1016/j.cub.2016.04.016.
- Salazar N, Arboleya S, Valdés L, et al. The human intestinal microbiome at extreme ages of life. Dietary intervention as a way to counteract alterations. *Front Genet.* 2014;5:406. doi:10.3389/fgene.2014.00406.
- Mariat D, Firmesse O, Levenez F, et al. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiol.* 2009;9:123. doi:10.1186/1471-2180-9-123.
- Urdaneta V, Casadesús J. Interactions between bacteria and bile salts in the gastrointestinal and hepatobiliary tracts. *Front Med.* 2017;4:163. doi:10.3389/fmed.2017.00163.
- Rowland I, Gibson G, Heinken A, et al. Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr.* 2018;57:1-24. doi:10.1007/s00394-017-1445-8.
- Macfarlane S, Macfarlane GT. Regulation of short-chain fatty acid production. *Proc Nutr Soc.* 2003;62:67-72. doi:10.1079/PNS2002207.
- Dalile B, Van Oudenhove L, Vervliet B, Verbeke K. The role of short-chain fatty acids in microbiota-gut-brain communication. *Nat Rev Gastroenterol Hepatol.* 2019;16:461-478. doi:10.1038/s41575-019-0157-3.
- Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. *Nat Rev Immunol.* 2016;16:341-352. doi:10.1038/nri.2016.42.
- Kitamoto S, Nagao-Kitamoto H, Kuffa P, Kamada N. Regulation of virulence: the rise and fall of gastrointestinal pathogens. *J Gastroenterol.* 2016;51:195-205. doi:10.1007/s00535-015-1141-5.
- Wang HX, Wang YP. Gut microbiota-brain axis. *Chin Med J.* 2016;129:2373-2380. doi:10.4103/0366-6999.190667.
- Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol.* 2015;28:203-209.
- Furness JB, Callaghan BP, Rivera LR, Cho H-J. The enteric nervous system and gastrointestinal innervation: integrated local and central control. *Adv Exp Med Biol.* 2014;817:39-71. doi:10.1007/978-1-4939-0897-4\_3.
- Mulak A, Bonaz B. Irritable bowel syndrome: a model of the brain-gut interactions. *Med Sci Monit.* 2004;10:RA55-RA62.
- Wood J, Alpers D, Andrews PL. Fundamentals of neurogastroenterology. *Gut.* 1999;45:II6-II16.
- O'Mahony SM, Hyland NP, Dinan TG, Cryan JF. Maternal separation as a model of brain-gut axis dysfunction. *Psychopharmacology.* 2011;214:71-88. doi:10.1007/s00213-010-2010-9.
- Furness JB. The enteric nervous system and neurogastroenterology. *Nat Rev Gastroenterol Hepatol.* 2012;9:286-294. doi:10.1038/nrgastro.2012.32.
- Kunze WAA, Furness JB, Bertrand PP, Bornstein JC. Intracellular recording from myenteric neurons of the guinea-pig ileum that respond to stretch. *J Physiol.* 1998;506:827-842. doi:10.1111/j.1469-7793.1998.827bv.x.
- Martin CR, Osadchiv V, Kalani A, Mayer EA. The brain-gut-microbiome axis. *Cell Mol Gastroenterol Hepatol.* 2018;6:133-148. doi:10.1016/j.jemgh.2018.04.003.
- Borre YE, O'Keefe GW, Clarke G, Stanton C, Dinan TG, Cryan JF. Microbiota and neurodevelopmental windows: implications for brain disorders. *Trends Mol Med.* 2014;20:509-518. doi:10.1016/j.molmed.2014.05.002.
- Kunze WA, Mao Y-K, Wang B, et al. Lactobacillus reuteri enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. *J Cell Mol Med.* 2009;13:2261-2270. doi:10.1111/j.1582-4934.2009.00686.x.
- Abrams GD, Bishop JE. Effect of the normal microbial flora on gastrointestinal motility. *Proc Soc Exp Biol Med.* 1967;126:301-304. doi:10.3181/00379727-126-32430.
- Barbara G, Stanghellini V, Brandi G, et al. Interactions between commensal bacteria and gut sensorimotor function in health and disease. *Am J Gastroenterol.* 2005;100:2560-2568. doi:10.1111/j.1572-0241.2005.00230.x.
- Holzer P, Farzi A. Neuropeptides and the microbiota-gut-brain axis. *Adv Exp Med Biol.* 2014;817:196-219. doi:10.1007/978-1-4939-0897-4\_9.
- Cani PD, Everard A, Duparc T. Gut microbiota, enteroendocrine functions and metabolism. *Curr Opin Pharmacol.* 2013;13:935-940. doi:10.1016/j.coph.2013.09.008.
- Waclawiková B, El Aidy S. Role of microbiota and tryptophan metabolites in the remote effect of intestinal inflammation on brain and depression. *Pharmacologicals.* 2018;11:E63. doi:10.3390/ph11030063.
- Strandwitz P. Neurotransmitter modulation by the gut microbiota. *Brain Res.* 2018;1693:128-133. doi:10.1016/j.brainres.2018.03.015.
- Lugo-Huitrón R, Ugalde Muñiz P, Pineda B, Pedraza-Chaverri J, Ríos C, Pérez-De La Cruz V. Quinolinic acid: an endogenous neurotoxin with multiple targets. *Oxid Med Cell Longev.* 2013;2013:104024. doi:10.1155/2013/104024.
- Le Floch N, Otten W, Merlot E. Tryptophan metabolism, from nutrition to potential therapeutic applications. *Amino Acids.* 2011;41:1195-1205. doi:10.1007/s00726-010-0752-7.
- O'Rourke L, Clarke G, Nolan A, et al. Tryptophan metabolic profile in term and preterm breast milk: implications for health. *J Nutr Sci.* 2018;7:e13.

54. Sainio EL, Pulkki K, Young SN. L-tryptophan: biochemical, nutritional and pharmacological aspects. *Amino Acids*. 1996;10:21-47. doi:10.1007/BF00806091.
55. Radwanski ER, Last RL. Tryptophan biosynthesis and metabolism: biochemical and molecular genetics. *Plant Cell*. 1995;7:921-934. doi:10.1105/tpc.7.7.921.
56. Slominski A, Semak I, Pisarchik A, Sweatman T, Szczesniowski A, Wortsman J. Conversion of L-tryptophan to serotonin and melatonin in human melanoma cells. *FEBS Lett*. 2002;511:102-106. doi:10.1016/S0014-5793(01)03319-1.
57. Palego L, Betti L, Rossi A, Giannaccini G. Tryptophan biochemistry: structural, nutritional, metabolic, and medical aspects in humans. *J Amino Acids*. 2016;2016:8952520. doi:10.1155/2016/8952520.
58. Roager HM, Licht TR. Microbial tryptophan catabolites in health and disease. *Nat Commun*. 2018;9:1-10. doi:10.1038/s41467-018-05470-4.
59. Jenkins TA, Nguyen JCD, Polglaze KE, Bertrand PP. Influence of tryptophan and serotonin on mood and cognition with a possible role of the gut-brain axis. *Nutrients*. 2016;8:56. doi:10.3390/nu810056.
60. Badawy AAB. Tryptophan availability for kynurenine pathway metabolism across the life span: control mechanisms and focus on aging, exercise, diet and nutritional supplements. *Neuropharmacology*. 2017;112:248-263. doi:10.1016/j.neuropharm.2015.11.015.
61. Kanai M, Funakoshi H, Takahashi H, et al. Tryptophan 2,3-dioxygenase is a key modulator of physiological neurogenesis and anxiety-related behaviour in mice. *Molec Brain*. 2009;2:8. doi:10.1186/1756-6606-2-8.
62. Jones SP, Franco NF, Varney B, et al. Expression of the kynurenine pathway in human peripheral blood mononuclear cells: implications for inflammatory and neurodegenerative disease. *PLoS ONE*. 2015;10:e0131389. doi:10.1371/journal.pone.0131389.
63. Ozaki Y, Edelstein MP, Duch DS. The actions of interferon and anti-inflammatory agents on induction of indoleamine 2,3-dioxygenase in human peripheral blood monocytes. *Biochem Biophys Res Commun*. 1987;144:1147-1153. doi:10.1016/0006-291X(87)91431-8.
64. Pfefferkorn ER, Rebhun S, Eckel M. Characterization of an indoleamine 2,3-dioxygenase induced by gamma-interferon in cultured human fibroblasts. *J Interferon Res*. 1986;6:267-279. doi:10.1089/jir.1986.6.267.
65. Currier AR, Ziegler MH, Riley MM, Babcock TA, Telbis VP, Carlin JM. Tumor necrosis factor-alpha and lipopolysaccharide enhance interferon-induced antichlamydia indoleamine dioxygenase activity independently. *J Interferon Cytokine Res*. 2000;20:369-376.
66. Li JS, Han Q, Fang J, Rizzi M, James AA, Li J. Biochemical mechanisms leading to tryptophan 2,3-dioxygenase activation. *Arch Insect Biochem Physiol*. 2007;64:74-87. doi:10.1002/arch.20159.
67. Chen Y, Guillemin GJ. Kynurenine pathway metabolites in humans: disease and healthy states. *Int J Tryptophan Res*. 2009;2:1-19. doi:10.4137/ijtr.s2097.
68. Dehghani M, Kazemi Shariat Panahi H, Guillemin GJ. Microorganisms, tryptophan metabolism, and kynurenine pathway: a complex interconnected loop influencing human health status [published online ahead of print June 19, 2019]. *Int J Tryptophan Res*. doi:10.1177/1178646919852996.
69. Nishizuka Y, Hayaishi O. Enzymic synthesis of niacin nucleotides from 3-hydroxyanthranilic acid in mammalian liver. *J Biol Chem*. 1963;238:483-485.
70. Bender DA. Effects of a dietary excess of leucine and of the addition of leucine and 2-oxo-isocaproate on the metabolism of tryptophan and niacin in isolated rat liver cells. *Br J Nutr*. 1989;61:629-640. doi:10.1079/bjn19890150.
71. Carpanese E, Moretto P, Filpa V, et al. Antagonism of ionotropic glutamate receptors attenuates chemical ischemia-induced injury in rat primary cultured myenteric ganglia. *PLoS ONE*. 2014;9:e113613. doi:10.1371/journal.pone.0113613.
72. Filpa V, Carpanese E, Marchet S, et al. Interaction between NMDA glutamatergic and nitric oxide pathways during in vitro ischemia and reperfusion. *Eur J Pharmacol*. 2015;750:123-131. doi:10.1016/j.ejphar.2015.01.021.
73. Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain: when physiology meets pathology. *Nat Rev Neurosci*. 2012;13:465-477. doi:10.1038/nrn3257.
74. Misztal M, Frankiewicz T, Parsons CG, Danysz W. Learning deficits induced by chronic intraventricular infusion of quinolinic acid: protection by MK-801 and memantine. *Eur J Pharmacol*. 1996;296:1-8. doi:10.1016/0014-2999(95)00682-6.
75. Tavares RG, Tasca CI, Santos CES, Wajner M, Souza DO, Dutra-Filho CS. Quinolinic acid inhibits glutamate uptake into synaptic vesicles from rat brain. *Neuroreport*. 2000;11:249-253. doi:10.1097/00001756-200002070-00005.
76. Tavares RG, Tasca CI, Santos CES, et al. Quinolinic acid stimulates synaptosomal glutamate release and inhibits glutamate uptake into astrocytes. *Neurochem Int*. 2002;40:621-627. doi:10.1016/S0197-0186(01)00133-4.
77. Guillemin GJ. Quinolinic acid, the inescapable neurotoxin. *FEBS J*. 2012;279:1356-1365. doi:10.1111/j.1742-4658.2012.08485.x.
78. Cruz VPD, La Carrillo-Mora P, Santamaría A. Quinolinic acid, an endogenous molecule combining excitotoxicity, oxidative stress and other toxic mechanisms. *Int J Tryptophan Res*. 2013;5:1-8. doi:10.4137/IJTR.S8158.
79. Myint AM. Kynurenines: from the perspective of major psychiatric disorders. *FEBS J*. 2012;279:1375-1385. doi:10.1111/j.1742-4658.2012.08551.x.
80. Guillemin GJ, Brew BJ. Implications of the kynurenine pathway and quinolinic acid in Alzheimer's disease. *Redox Rep*. 2002;7:199-206. doi:10.1179/13510002125000550.
81. Zinger A, Barcia C, Herrero MT, Guillemin GJ. The involvement of neuroinflammation and kynurenine pathway in Parkinson's disease. *Parkinsons Dis*. 2011;2011:716859. doi:10.4061/2011/716859.
82. Sundaram G, Brew BJ, Jones SP, Adams S, Lim CK, Guillemin GJ. Quinolinic acid toxicity on oligodendroglial cells: relevance for multiple sclerosis and therapeutic strategies. *J Neuroinflammation*. 2014;11:204. doi:10.1186/s12974-014-0204-5.
83. Bagasrawala I, Zecevic N, Radonjic NV. N-methyl D-aspartate receptor antagonist kynurenic acid affects human cortical development. *Front Neurosci*. 2016;10:435. doi:10.3389/fnins.2016.00435.
84. Hilmas C, Pereira EFR, Alkondon M, Rassoulpour A, Schwarcz R, Albuquerque EX. The brain metabolite kynurenic acid inhibits  $\alpha 7$  nicotinic receptor activity and increases non- $\alpha 7$  nicotinic receptor expression: physiopathological implications. *J Neurosci*. 2001;21:7463-7473. doi:10.1523/jneurosci.21-19-07463.2001.
85. Wang J, Simonavicius N, Wu X, et al. Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. *J Biol Chem*. 2006;281:22021-22028. doi:10.1074/jbc.M603503200.
86. O'Farrell K, Harkin A. Stress-related regulation of the kynurenine pathway: relevance to neuropsychiatric and degenerative disorders. *Neuropharmacology*. 2017;112:307-323. doi:10.1016/j.neuropharm.2015.12.004.
87. Strasser B, Becker K, Fuchs D, Gostner JM. Kynurenine pathway metabolism and immune activation: peripheral measurements in psychiatric and comorbid conditions. *Neuropharmacology*. 2017;112:286-296. doi:10.1016/j.neuropharm.2016.02.030.
88. Mardinoglu A, Shoaie S, Bergental M, et al. The gut microbiota modulates host amino acid and glutathione metabolism in mice. *Mol Syst Biol*. 2015;11:834. doi:10.15252/msb.20156487.
89. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci*. 2012;13:701-712. doi:10.1038/nrn3346.
90. Wikoff WR, Anfora AT, Liu J, et al. Metabolomics analysis reveals large effects of gut microbiota on mammalian blood metabolites. *Proc Natl Acad Sci U S A*. 2009;106:3698-3703. doi:10.1073/pnas.0812874106.
91. Desbonnet L, Garrett L, Clarke G, Bienenstock J, Dinan TG. The probiotic *Bifidobacterium infantis*: an assessment of potential antidepressant properties in the rat. *J Psychiatr Res*. 2008;43:164-174. doi:10.1016/j.jpsychires.2008.03.009.
92. Martin-Gallausiaux C, Larraufie P, Jarry A, et al. Butyrate produced by commensal bacteria down-regulates indoleamine 2,3-dioxygenase 1 (IDO-1) expression via a dual mechanism in human intestinal epithelial cells. *Front Immunol*. 2018;9:2838. doi:10.3389/fimmu.2018.02838.
93. Freewan M, Rees MD, Plaza TSS, et al. Human indoleamine 2,3-dioxygenase is a catalyst of physiological heme peroxidase reactions: implications for the inhibition of dioxygenase activity by hydrogen peroxide. *J Biol Chem*. 2013;288:1548-1567. doi:10.1074/jbc.M112.410993.
94. Valladares R, Bojilova L, Potts AH, et al. *Lactobacillus johnsonii* inhibits indoleamine 2,3-dioxygenase and alters tryptophan metabolite levels in Bio-Breeding rats. *FASEB J*. 2013;27:1711-1720. doi:10.1096/fj.12-223339.
95. Forrest CM, McNair K, Pisar M, Khalil OS, Darlington LG, Stone TW. Altered hippocampal plasticity by prenatal kynurenine administration, kynurenine-3-monooxygenase (KMO) deletion or galantamine. *Neuroscience*. 2015;310:91-105. doi:10.1016/j.neuroscience.2015.09.022.
96. Khalil OS, Pisar M, Forrest CM, Vincenten MCJ, Darlington LG, Stone TW. Prenatal inhibition of the kynurenine pathway leads to structural changes in the hippocampus of adult rat offspring. *European J Neurosci*. 2014;39:1558-1571. doi:10.1111/ejn.12535.
97. Pisar M, Forrest CM, Khalil OS, et al. Modified neocortical and cerebellar protein expression and morphology in adult rats following prenatal inhibition of the kynurenine pathway. *Brain Res*. 2014;1576:1-17. doi:10.1016/j.brainres.2014.06.016.
98. Mawe GM, Hoffman JM. Serotonin signalling in the gut-functions, dysfunctions and therapeutic targets. *Nat Rev Gastroenterol Hepatol*. 2013;10:473-486. doi:10.1038/nrgastro.2013.105.
99. Camilleri M. Serotonin in the gastrointestinal tract. *Curr Opin Endocrinol Diabetes Obes*. 2009;16:53-59. doi:10.1097/MED.0b013e32831e9c8e.
100. Richard DM, Dawes MA, Mathias CW, Acheson A, Hill-Kapturczak N, Dougherty DM. L-tryptophan: basic metabolic functions, behavioural research and therapeutic indications. *Int J Tryptophan Res*. 2009;2:45-60. doi:10.4137/ijtr.s2129.
101. El-Merahbi R, Löffler M, Mayer A, Sumara G. The roles of peripheral serotonin in metabolic homeostasis. *FEBS Lett*. 2015;589:1728-1734. doi:10.1016/j.febslet.2015.05.054.
102. O'Mahony SM, Clarke G, Borre YE, Dinan TG, Cryan JF. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behav Brain Res*. 2015;277:32-48. doi:10.1016/j.bbr.2014.07.027.

103. Tordjman S, Chokron S, Delorme R, et al. Send orders for reprints to reprints@benthamscience.ac melatonin: pharmacology, functions and therapeutic benefits. *Curr Neuropsychopharmacol*. 2017;15:434-443. doi:10.2174/1570159X14666161228122.
104. Nichols DE, Nichols CD. Serotonin receptors. *Chem Rev*. 2008;108:1614-1641. doi:10.1021/cr078224a.
105. Özoğul F. Production of biogenic amines by *Morganella morganii*, *Klebsiella pneumoniae* and *Hafnia alvei* using a rapid HPLC method. *Eur Food Res Technol*. 2004;219:465-469. doi:10.1007/s00217-004-0988-0.
106. Özoğul F, Kuley E, Özoğul Y, Özoğul İ. The function of lactic acid bacteria on biogenic amines production by food-borne pathogens in arginine decarboxylase broth consequently, the function of LAB strains on ammonia (AMN) and BAs production by gram negative and positive FBP varied depending on. *Food Sci Technol Res*. 2012;18:795-804.
107. Shishov VA, Kirovskaya TA, Kudrin VS, Oleskin AV. Amine neuromediators, their precursors, and oxidation products in the culture of *Escherichia coli* k-12. *Appl Biochem Microbiol*. 2009;45:494-497. doi:10.1134/S0003683809050068.
108. Reigstad CS, Salmonson CE, Rainey JF, et al. Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *FASEB J*. 2015;29:1395-1403. doi:10.1096/fj.14-259598.
109. Hata T, Asano Y, Yoshihara K, et al. Regulation of gut luminal serotonin by commensal microbiota in mice. *PLoS ONE*. 2017;12:e0180745. doi:10.1371/journal.pone.0180745.
110. Cao Y-N, Feng L-J, Liu Y-Y, et al. Effect of *Lactobacillus rhamnosus* GG supernatant on serotonin transporter expression in rats with post-infectious irritable bowel syndrome. *World J Gastroenterol*. 2018;24:338-350. doi:10.3748/wjg.v24.i3.338.
111. Alemi F, Poole DP, Chiu J, et al. The receptor TGR5 mediates the prokinetic actions of intestinal bile acids and is required for normal defecation in mice. *Gastroenterology*. 2013;144:145-154. doi:10.1053/j.gastro.2012.09.055.
112. Golubeva AV, Joyce SA, Moloney G, et al. Microbiota-related changes in bile acid & tryptophan metabolism are associated with gastrointestinal dysfunction in a mouse model of autism. *EbioMedicine*. 2017;24:166-178. doi:10.1016/j.ebiom.2017.09.020.
113. Williams BB, Van Benschoten AH, Cimermancic P, et al. Discovery and characterization of gut microbiota decarboxylases that can produce the neurotransmitter tryptamine. *Cell Host Microbe*. 2014;16:495-503. doi:10.1016/j.chom.2014.09.001.
114. Marcobal A, Kashyap PC, Nelson TA, et al. A metabolomic view of how the human gut microbiota impacts the host metabolome using humanized and gnotobiotic mice. *ISME J*. 2013;7:1933-1943. doi:10.1038/ismej.2013.89.
115. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*. 2010;464:59-65. doi:10.1038/nature08821.
116. Sagheddu V, Patron V, Miragoli F, Puglisi E, Morelli L. Infant early gut colonization by Lachnospiraceae: high frequency of *Ruminococcus gnavus*. *Front Pediatr*. 2016;4:57. doi:10.3389/fped.2016.00057.
117. Yanofsky C, Horn V, Rollick P. Physiological studies of tryptophan transport and tryptophanase operon induction in *Escherichia coli*. *J Bacteriol*. 1991;173:6009-6017. doi:10.1128/jb.173.19.6009-6017.1991.
118. Zelante T, Iannitti RG, Cunha C, et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity*. 2013;39:372-385. doi:10.1016/j.immuni.2013.08.003.
119. Whitehead TR, Price NP, Drake HL, Cotta MA. Catabolic pathway for the production of skatole and indoleacetic acid by the acetogen *Clostridium drakei*, *Clostridium scatologenes*, and swine manure. *Appl Environ Microbiol*. 2008;74:1950-1953. doi:10.1128/AEM.02458-07.
120. Cook KL, Rothrock MJ, Loughrin JH, Doerner KC. Characterization of skatole-producing microbial populations in enriched swine lagoon slurry. *FEMS Microbiol Ecol*. 2007;60:329-340. doi:10.1111/j.1574-6941.2007.00299.x.
121. Yokoyama MT, Carlson JR. Microbial metabolites of tryptophan in the intestinal tract with special reference to skatole. *Am J Clin Nutr*. 1979;32:173-178. doi:10.1093/ajcn/32.1.173.
122. Smith EA, Macfarlane GT. Formation of phenolic and indolic compounds by anaerobic bacteria in the human large intestine. *Micro Ecol*. 1997;33:180-188. doi:10.1007/s002489900020.
123. Hubbard TD, Murray IA, Perdew GH. Special section on drug metabolism and the microbiome – minireview indole and tryptophan metabolism: endogenous and dietary routes to ah receptor activation. *Drug Metab Dispos*. 2015;43:1522-1535. doi:10.1124/dmd.115.064246.
124. Stockinger B, Meglio P, Di Gialitakis M, Duarte JH. The aryl hydrocarbon receptor: multitasking in the immune system. *Annu Rev Immunol*. 2014;32:403-432. doi:10.1146/annurev-immunol-032713-120245.
125. Darkoh C, Chappell C, Gonzales C, Okhuysen P. A rapid and specific method for the detection of indole in complex biological samples. *Appl Environ Microbiol*. 2015;81:8093-8097. doi:10.1128/AEM.02787-15.
126. Pavlova T, Viola V, Bienertova-Vasku J, et al. Urinary intermediates of tryptophan as indicators of the gut microbial metabolism. *Anal Chim Acta*. 2017;987:72-80. doi:10.1016/j.aca.2017.08.022.
127. Chelakkot C, Ghim J, Ryu SH. Mechanisms regulating intestinal barrier integrity and its pathological implications. *Exp Mol Med*. 2018;50:103. doi:10.1038/s12276-018-0126-x.
128. Arike L, Seiman A, van der Post S, et al. Protein turnover in epithelial cells and mucus along the gastrointestinal tract is coordinated by the spatial location and microbiota. *Cell Rep*. 2020;30:1077-1087.e3. doi:10.1016/j.celrep.2019.12.068.
129. Abrams GD, Bauer H, Sprinz H. Influence of the normal flora on mucosal morphology and cellular renewal in the ileum. A comparison of germ-free and conventional mice. *Lab Invest*. 1963;12:355-364.
130. Yan F, Cao H, Cover TL, et al. Colon-specific delivery of a probiotic-derived soluble protein ameliorates intestinal inflammation in mice through an EGFR-dependent mechanism. *J Clin Invest*. 2011;121:2242-2253. doi:10.1172/JCI44031.
131. Yan F, Polk DB. Probiotic bacterium prevents cytokine-induced apoptosis in intestinal epithelial cells. *J Biol Chem*. 2002;277:50959-50965. doi:10.1074/jbc.M207050200.
132. Hughes KR, Harnisch LC, Alcon-Giner C, et al. Bifidobacterium breve reduces apoptotic epithelial cell shedding in an exopolysaccharide and MyD88-dependent manner. *Open Biol*. 2017;7:160155. doi:10.1098/rsob.160155.
133. Ieronimo G, Palmisano G, Maspero A, et al. A novel synthesis of N-hydroxy-3-aryloindoles and 3-aryloindoles. *Org Biomol Chem*. 2018;16:6853-6859. doi:10.1039/C8OB01471J.
134. Li G, Young KD. Indole production by the tryptophanase TnaA in *Escherichia coli* is determined by the amount of exogenous tryptophan. *Microbiology*. 2013;159:402-410. doi:10.1099/mic.0.064139-0.
135. Scapinello L, Maspero A, Tollari S, Palmisano G, Nicholas KM, Penoni A. A direct, regioselective and atom-economical synthesis of 3-aryl-N-hydroxy-5-nitroindoles by cycloaddition of 4-nitronitrosobenzene with alkynes. *J Vis Exp*. 2020;155:e60201. doi:10.3791/60201.
136. Sartor B. Intestinal bacterial biofilms modulate mucosal immune responses. *J Immunol Sci*. 2018;2:13-18. doi:10.29245/2578-3009/2018/2.1122.
137. Bansal T, Alaniz RC, Wood TK, Jayaraman A. The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. *Proc Natl Acad Sci U S A*. 2010;107:228-233. doi:10.1073/pnas.0906112107.
138. Shimada Y, Kinoshita M, Harada K, et al. Commensal bacteria-dependent indole production enhances epithelial barrier function in the colon. *PLoS ONE*. 2013;8:e80604. doi:10.1371/journal.pone.0080604.
139. Wlodarska M, Luo C, Kolde R, et al. Indoleacrylic acid produced by commensal *Peptostreptococcus* species suppresses inflammation. *Cell Host Microbe*. 2017;22:25-37.e6. doi:10.1016/j.chom.2017.06.007.
140. Dodd D, Spitzer MH, Van Treuren W, et al. A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites. *Nature*. 2017;551:648-652. doi:10.1038/nature24661.
141. Jennis M, Cavanaugh CR, Leo GC, Mabus JR, Lenhard J, Hornby PJ. Microbiota-derived tryptophan indoles increase after gastric bypass surgery and reduce intestinal permeability in vitro and in vivo. *Neurogastroenterol Motil*. 2018;30:e13178. doi:10.1111/nmo.13178.
142. Venkatesh M, Mukherjee S, Wang H, et al. Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and toll-like receptor 4. *Immunity*. 2014;41:296-310. doi:10.1016/j.immuni.2014.06.014.
143. Caputi V, Giron MC. Microbiome-gut-brain axis and toll-like receptors in Parkinson's disease. *Int J Mol Sci*. 2018;19:1689. doi:10.3390/ijms19061689.
144. Kawasaki T, Kawai T. Toll-like receptor signaling pathways. *Front Immunol*. 2014;5:461. doi:10.3389/fimmu.2014.00461.
145. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell*. 2010;140:805-820. doi:10.1016/j.cell.2010.01.022.
146. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell*. 2004;118:229-241. doi:10.1016/j.cell.2004.07.002.
147. Cario E, Gerken G, Podolsky DK. Toll-like receptor 2 enhances ZO-1-associated intestinal epithelial barrier integrity via protein kinase C. *Gastroenterology*. 2004;127:224-238. doi:10.1053/j.gastro.2004.04.015.
148. Gareau MG, Silva MA, Perdew MH. Pathophysiological mechanisms of stress-induced intestinal damage. *Curr Mol Med*. 2008;8:274-281. doi:10.2174/156652408784533760.
149. Maes M, Leunis JC. Normalization of leaky gut in chronic fatigue syndrome (CFS) is accompanied by a clinical improvement: effects of age, duration of illness and the translocation of LPS from gram-negative bacteria. *Neuro Endocrinol Lett*. 2008;29:902-910.
150. Sarkar A, Lehto SM, Harty S, Dinan TG, Cryan JF, Burnet PJ. Psychobiotics and the manipulation of bacteria-gut-brain signals. *Trends Neurosci*. 2016;39:763-781. doi:10.1016/j.tins.2016.09.002.
151. Sternini C, Anselmi L, Rozengurt E. Enteroendocrine cells: a site of 'taste' in gastrointestinal chemosensing. *Curr Opin Endocrinol Diabetes Obes*. 2008;15:73-78. doi:10.1097/MED.0b013e3282f43a73.

152. Gribble FM, Reimann F. Enteroendocrine cells: chemosensors in the intestinal epithelium. *Annu Rev Physiol*. 2016;78:277-299. doi:10.1146/annurev-physiol-021115-105439.
153. Fothergill LJ, Furness JB. Diversity of enteroendocrine cells investigated at cellular and subcellular levels: the need for a new classification scheme. *Histochem Cell Biol*. 2018;150:693-702. doi:10.1007/s00418-018-1746-x.
154. Gunawardene AR, Corfe BM, Staton CA. Classification and functions of enteroendocrine cells of the lower gastrointestinal tract. *Int J Exp Pathol*. 2011;92:219-231. doi:10.1111/j.1365-2613.2011.00767.x.
155. Bellono NW, Bayrer JR, Leitch DB, et al. Enterochromaffin cells are gut chemosensors that couple to sensory neural pathways. *Cell*. 2017;170:185-198.e16. doi:10.1016/j.cell.2017.05.034.
156. Tsubouchi S, Leblond CP. Migration and turnover of entero-endocrine and caveolated cells in the epithelium of the descending colon, as shown by radioautography after continuous infusion of 3H-thymidine into mice. *Am J Anat*. 1979;156:431-451. doi:10.1002/aja.1001560403.
157. Yu Y, Yang W, Li Y, Cong Y. Enteroendocrine cells: sensing gut microbiota and regulating inflammatory bowel diseases. *Inflamm Bowel Dis*. 2020;26:11-20. doi:10.1093/ibd/izz217.
158. Habib AM, Richards P, Rogers CJ, Reimann F, Gribble FM. Co-localisation and secretion of glucagon-like peptide 1 and peptide YY from primary cultured human L cells. *Diabetologia*. 2013;56:1413-1416. doi:10.1007/s00125-013-2887-z.
159. Habib AM, Richards P, Cairns LS, et al. Overlap of endocrine hormone expression in the mouse intestine revealed by transcriptional profiling and flow cytometry. *Endocrinology*. 2012;153:3054-3065. doi:10.1210/en.2011-2170.
160. Steinert RE, Feinle-Bisset C, Asarian L, Horowitz M, Beglinger C, Geary N. Ghrelin, CCK, GLP-1, and PYY(3-36): secretory controls and physiological roles in eating and Glycemia in health, obesity, and after RYGB. *Physiol Rev*. 2017;97:411-463. doi:10.1152/physrev.00031.2014.
161. De Silva A, Bloom SR. Gut hormones and appetite control: a focus on PYY and GLP-1 as therapeutic targets in obesity. *Gut Liver*. 2012;6:10-20. doi:10.5009/gnl.2012.6.1.10.
162. Richards P, Parker HE, Adriaenssens AE, et al. Identification and characterization of GLP-1 receptor-expressing cells using a new transgenic mouse model. *Diabetes*. 2014;63:1224-1233. doi:10.2337/db13-1440.
163. Kaelberer MM, Buchanan KL, Klein ME, et al. A gut-brain neural circuit for nutrient sensory transduction. *Science (New York, NY)*. 2018;361:eaat5236. doi:10.1126/science.aat5236.
164. Engelstoft MS, Egerod KL, Holst B, Schwartz TW. A gut feeling for obesity: 7TM sensors on enteroendocrine cells. *Cell Metab*. 2008;8:447-449. doi:10.1016/j.cmet.2008.11.004.
165. Chimere C, Emery E, Summers DK, Keyser U, Gribble FM, Reimann F. Bacterial metabolite indole modulates incretin secretion from intestinal enteroendocrine L cells. *Cell Rep*. 2014;9:1202-1208. doi:10.1016/j.celrep.2014.10.032.
166. Aziz A, Anderson GH. Exendin-4, a GLP-1 receptor agonist, interacts with proteins and their products of digestion to suppress food intake in rats. *J Nutr*. 2003;133:2326-2330. doi:10.1093/jn/133.7.2326.
167. Popoff MR, Poulain B. Bacterial toxins and the nervous system: neurotoxins and multipotential toxins interacting with neuronal cells. *Toxins*. 2010;2:683-737. doi:10.3390/toxins2040683.
168. Peregrin AT, Ahlman H, Jodal M, Lundgren O. Involvement of serotonin and calcium channels in the intestinal fluid secretion evoked by bile salt and cholera toxin. *Br J Pharmacol*. 1999;127:887-894. doi:10.1038/sj.bjp.0702615.
169. Stephens RL, Tache Y. Intracisternal injection of a TRH analogue stimulates gastric luminal serotonin release in rats. *Am J Physiol*. 1989;256:G377-G383. doi:10.1152/ajpgi.1989.256.2.G377.
170. Grøndahl ML, Unmack MA, Ragnarsdóttir HB, Hansen MB, Olsen JE, Skadhauge E. Effects of nitric oxide in 5-hydroxytryptamine-, cholera toxin-, enterotoxigenic *Escherichia coli*- and *Salmonella typhimurium*-induced secretion in the porcine small intestine. *Comp Biochem Physiol A Mol Integr Physiol*. 2005;141:476-484. doi:10.1016/j.cbpb.2005.07.001.
171. Yano JM, Yu K, Donaldson GP, et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*. 2015;161:264-276. doi:10.1016/j.cell.2015.02.047.
172. Hansen MB, Witte A-B. The role of serotonin in intestinal luminal sensing and secretion. *Acta Physiol (Oxford, England)*. 2008;193:311-323. doi:10.1111/j.1748-1716.2008.01870.x.
173. Bogunovic M, Davé SH, Tilstra JS, et al. Enteroendocrine cells express functional Toll-like receptors. *Am J Physiol Gastrointest Liver Physiol*. 2007;292:G1770-G1783. doi:10.1152/ajpgi.00249.2006.
174. Kidd M, Gustafsson BI, Drozdov I, Modlin IM. IL1 $\beta$ - and LPS-induced serotonin secretion is increased in EC cells derived from Crohn's disease. *Neurogastroenterol Motil*. 2009;21:439-450. doi:10.1111/j.1365-2982.2008.01210.x.
175. Gershon MD, Tack J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. *Gastroenterology*. 2007;132:397-414. doi:10.1053/j.gastro.2006.11.002.
176. Xue J, Askwith C, Javed NH, Cooke HJ. Autonomic nervous system and secretion across the intestinal mucosal surface. *Auton Neurosci*. 2007;133:55-63. doi:10.1016/j.autneu.2007.02.001.
177. Gershon MD. 5-Hydroxytryptamine (serotonin) in the gastrointestinal tract. *Curr Opin Endocrinol Diabetes Obes*. 2013;20:14-21. doi:10.1097/MED.0b013e32835bc703.
178. Biaggini K, Barbey C, Borrel V, Feuilletoy M, Déchelotte P, Connil N. The pathogenic potential of *Pseudomonas fluorescens* MFN1032 on enterocytes can be modulated by serotonin, substance P and epinephrine. *Arch Microbiol*. 2015;197:983-990. doi:10.1007/s00203-015-1135-y.
179. Lauder AP, Roche AM, Sherrill-Mix S, et al. Comparison of placenta samples with contamination controls does not provide evidence for a distinct placenta microbiota [published online ahead of print June 23, 2016]. *Microbiome*. doi:10.1186/s40168-016-0172-3.
180. Powell N, Walker MM, Talley NJ. The mucosal immune system: master regulator of bidirectional gut-brain communications. *Nat Rev Gastroenterol Hepatol*. 2017;14:143-159. doi:10.1038/nrgastro.2016.191.
181. Mayer L. Mucosal immunity and gastrointestinal antigen processing. *J Pediatr Gastroenterol Nutr*. 2000;30:S4-S12. doi:10.1097/00005176-200001001-00002.
182. Jung C, Hugot J-P, Barreau F. Peyer's patches: the immune sensors of the intestine. *Int J Inflamm*. 2010;2010:823710. doi:10.4061/2010/823710.
183. Pabst O, Mowat AM. Oral tolerance to food protein. *Mucosal Immunol*. 2012;5:232-239. doi:10.1038/mi.2012.4.
184. Staggs AJ. Intestinal dendritic cells in health and gut inflammation. *Front Immunol*. 2018;9:2883. doi:10.3389/fimmu.2018.02883.
185. Commins SP. Mechanisms of oral tolerance. *Pediatr Clin North Am*. 2015;62:1523-1529. doi:10.1016/j.pcl.2015.07.013.
186. Chandran P, Sathaporn S, Robins A, Eremin O. Inflammatory bowel disease: dysfunction of GALT and gut bacterial flora (II). *Surgeon*. 2003;1:125-136. doi:10.1016/S1479-666X(03)80091-4.
187. Quigley EM. Probiotics in functional gastrointestinal disorders: what are the facts? *Curr Opin Pharmacol*. 2008;8:704-708. doi:10.1016/j.coph.2008.08.007.
188. Gordon HA. Morphological and physiological characterization of germfree life. *Ann NY Acad Sci*. 1959;78:208-220. doi:10.1111/j.1749-6632.1959.tb53104.x.
189. Beaver MH, Wostmann BS. Histamine and 5-hydroxytryptamine in the intestinal tract of germ-free animals, animals harbouring one microbial species and conventional animals. *Br J Pharmacol Chemother*. 1962;19:385-393. doi:10.1111/j.1476-5381.1962.tb01443.x.
190. Smith CJ, Emge JR, Berzins K, et al. Probiotics normalize the gut-brain-microbiota axis in immunodeficient mice. *Am J Physiol Gastrointest Liver Physiol*. 2014;307:G793-G802. doi:10.1152/ajpgi.00238.2014.
191. Mikkelsen HB, Garbarsch C, Tranum-Jensen J, Thuneberg L. Macrophages in the small intestinal muscularis externa of embryos, newborn and adult germ-free mice. *J Mol Histol*. 2004;35:377-387.
192. Glaister JR. Factors affecting the lymphoid cells in the small intestinal epithelium of the mouse. *Int Arch Allergy Appl Immunol*. 1973;45:719-730. doi:10.1159/000231071.
193. Umesaki Y, Okada Y, Matsumoto S, Imaoka A, Setoyama H. Segmented filamentous bacteria are indigenous intestinal bacteria that activate intraepithelial lymphocytes and induce MHC class II molecules and fucosyl asialo GM1 glycolipids on the small intestinal epithelial cells in the ex-germ-free mouse. *Microbiol Immunol*. 1995;39:555-562. doi:10.1111/j.1348-0421.1995.tb02242.x.
194. Di Mauro A, Neu J, Riezzo G, et al. Gastrointestinal function development and microbiota. *Ital J Pediatr*. 2013;39:15. doi:10.1186/1824-7288-39-15.
195. Atarashi K, Tanoue T, Oshima K, et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature*. 2013;500:232-236. doi:10.1038/nature12331.
196. Keszthelyi D, Troost FJ, Masclee AAM. Understanding the role of tryptophan and serotonin metabolism in gastrointestinal function. *Neurogastroenterol Motil*. 2009;21:1239-1249. doi:10.1111/j.1365-2982.2009.01370.x.
197. Gao J, Xu K, Liu H, et al. Impact of the gut microbiota on intestinal immunity mediated by tryptophan metabolism. *Front Cell Infect Microbiol*. 2018;8:13. doi:10.3389/fcimb.2018.00013.
198. Gaffen SL, Jain R, Garg AV, Cua D. IL-23-IL-17 immune axis: discovery, mechanistic understanding, and clinical testing. *Nat Rev Immunol*. 2014;14:585-600. doi:10.1038/NRI3707.
199. Tiszlavicz Z, Németh B, Fülöp F, et al. Different inhibitory effects of kynurenic acid and a novel kynurenic acid analogue on tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) production by mononuclear cells, HMGB1 production by monocytes and HNP1-3 secretion by neutrophils. *Naunyn-Schmiedeberg Arch Pharmacol*. 2011;383:447-455. doi:10.1007/s00210-011-0605-2.
200. Mándi Y, Endrész V, Mosolygó T, et al. The opposite effects of kynurenic acid and different kynurenic acid analogs on tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) production and tumor necrosis factor-stimulated gene-6 (TSG-6) expression. *Front Immunol*. 2019;10:1406. doi:10.3389/fimmu.2019.01406.
201. Kaszaki J, Palásthy Z, Érczes D, et al. Kynurenic acid inhibits intestinal hypermotility and xanthine oxidase activity during experimental colon obstruction

- in dogs. *Neurogastroenterol Motil.* 2008;20:53-62. doi:10.1111/j.1365-2982.2007.00989.x.
202. Moroni F, Fossati S, Chiarugi A, Cozzi A. Kynurenic acid actions in brain and periphery. *Int Congr Ser.* 2007;1304:305-313. doi:10.1016/j.ics.2007.07.016.
203. Kiank C, Zeden JP, Drude S, et al. Psychological stress-induced, IDO1-dependent tryptophan catabolism: implications on immunosuppression in mice and humans. *PLoS ONE.* 2010;5:e11825. doi:10.1371/journal.pone.0011825.
204. Melillo G, Cox GW, Biragyn A, Sheffler LA, Varesio L. Regulation of nitric-oxide synthase mRNA expression by interferon- $\gamma$  and picolinic acid. *J Biol Chem.* 1994;269:8128-8133.
205. Varesio L, Clayton M, Blasi E, Ruffman R, Radzioch D. Picolinic acid, a catabolite of tryptophan, as the second signal in the activation of IFN- $\gamma$ -primed macrophages. *J Immunol.* 1990;145:4265-4271.
206. Bosco MC, Rapisarda A, Massazza S, Melillo G, Young H, Varesio L. The tryptophan catabolite picolinic acid selectively induces the chemokines macrophage inflammatory protein-1 $\alpha$  and -1 $\beta$  in macrophages. *J Immunol.* 2000;164:3283-3291. doi:10.4049/jimmunol.164.6.3283.
207. Cai S, Sato K, Shimizu T, et al. Antimicrobial activity of picolinic acid against extracellular and intracellular Mycobacterium avium complex and its combined activity with clarithromycin, rifampicin and fluoroquinolones. *J Antimicrob Chemother.* 2006;57:85-93. doi:10.1093/jac/dki418.
208. Shimizu T, Tomioka H. Activity of picolinic acid in combination with the anti-protozoal drug quinacrine against Mycobacterium avium complex. *Antimicrob Agents Chemother.* 2006;50:3186-3188. doi:10.1128/AAC.01510-05.
209. Xu K, Liu H, Bai M, Gao J, Wu X, Yin Y. Redox properties of tryptophan metabolism and the concept of tryptophan use in pregnancy. *Int J Mol Sci.* 2017;18:1595. doi:10.3390/ijms18071595.
210. Oxenkrug GF. Metabolic syndrome, age-associated neuroendocrine disorders, and dysregulation of tryptophan: kynurenine metabolism. *Ann NY Acad Sci.* 2010;1199:1-14. doi:10.1111/j.1749-6632.2009.05356.x.
211. Stockinger B, Omenetti S. The dichotomous nature of T helper 17 cells. *Nat Rev Immunol.* 2017;17:535-544. doi:10.1038/nri.2017.50.
212. Lee JS, Cella M, McDonald KG, et al. AHR drives the development of gut ILC22 cells and postnatal lymphoid tissues via pathways dependent on and independent of Notch. *Nature Immunol.* 2012;13:144-152. doi:10.1038/ni.2187.
213. Kiss EA, Vonarbourg C, Kopfmann S, et al. Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles. *Science.* 2011;334:1561-1565. doi:10.1126/science.1214914.
214. Kimura A, Naka T, Nakahama T, et al. Aryl hydrocarbon receptor in combination with Stat1 regulates LPS-induced inflammatory responses. *J Exp Med.* 2009;206:2027-2035. doi:10.1084/jem.20090560.
215. Nguyen NT, Kimura A, Nakahama T, et al. Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. *Proc Natl Acad Sci U S A.* 2010;107:19961-19966. doi:10.1073/pnas.1014465107.
216. Quintana FJ, Murugaiyan G, Farez MF, et al. An endogenous aryl hydrocarbon receptor ligand acts on dendritic cells and T cells to suppress experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A.* 2010;107:20768-20773. doi:10.1073/pnas.1009201107.
217. Zindl CL, Lai JF, Lee YK, et al. IL-22-producing neutrophils contribute to antimicrobial defense and restitution of colonic epithelial integrity during colitis. *Proc Natl Acad Sci U S A.* 2013;110:12768-12773. doi:10.1073/pnas.1300318110.
218. Cervantes-Barragan L, Chai JN, Tianero MD, et al. Lactobacillus reuteri induces gut intraepithelial CD4+CD8 $\alpha\alpha$ + T cells. *Science.* 2017;357:806-810. doi:10.1126/science.aah5825.
219. Gao K, Mu C, Farzi A, Zhu W. Tryptophan metabolism: a link between the gut microbiota and brain. *Adv Nutr.* 2020;11:709-723. doi:10.1093/advances/nmz127.
220. Orhan F, Bhat M, Sandberg K, et al. Tryptophan metabolism along the kynurenine pathway downstream of toll-like receptor stimulation in peripheral monocytes. *Scand J Immunol.* 2016;84:262-271. doi:10.1111/sji.12479.
221. Vermeulen W, De Man JG, Pelckmans PA, De Winter BY. Neuroanatomy of lower gastrointestinal pain disorders. *World J Gastroenterol.* 2014;20:1005-1020. doi:10.3748/wjg.v20.i4.1005.
222. Furness JB, Jones C, Nurgali K, Clerc N. Intrinsic primary afferent neurons and nerve circuits within the intestine. *Prog Neurobiol.* 2004;72:143-164. doi:10.1016/j.pneurobio.2003.12.004.
223. Bistoletti M, Caputi V, Baranzini N, et al. Antibiotic treatment-induced dysbiosis differently affects BDNF and TrkB expression in the brain and in the gut of juvenile mice. *PLoS ONE.* 2019;14:e0212856. doi:10.1371/journal.pone.0212856.
224. Ceccotti C, Giaroni C, Bistoletti M, Viola M, Crema F, Terova G. Neurochemical characterization of myenteric neurons in the juvenile gilthead sea bream (*Sparus aurata*) intestine. *PLoS ONE.* 2018;13:e0201760. doi:10.1371/journal.pone.0201760.
225. Kendig DM, Grider JR. Serotonin and colonic motility. *Neurogastroenterol Motil.* 2015;27:899-905. doi:10.1111/nmo.12617.
226. Spencer NJ. Constitutively active 5-HT receptors: an explanation of how 5-HT antagonists inhibit gut motility in species where 5-HT is not an enteric neurotransmitter? *Front Cell Neurosci.* 2015;9:487. doi:10.3389/fncel.2015.00487.
227. Giaroni C. Purinergic signalling and development of the autonomic nervous system. *Auton Neurosci.* 2015;191:67-77. doi:10.1016/j.autneu.2015.04.009.
228. Filpa V, Carpanese E, Marchet S, et al. Nitric oxide regulates homeoprotein OTX1 and OTX2 expression in the rat myenteric plexus after intestinal ischemia-reperfusion injury. *Am J Physiol Gastrointest Liver Physiol.* 2017;312:G374-G389. doi:10.1152/ajpgi.00386.2016.
229. Filpa V, Bistoletti M, Caon I, et al. Changes in hyaluronan deposition in the rat myenteric plexus after experimentally-induced colitis. *Sci Rep.* 2017;7:17644. doi:10.1038/s41598-017-18020-7.
230. Bistoletti M, Micheloni G, Baranzini N, et al. Homeoprotein OTX1 and OTX2 involvement in rat myenteric neuron adaptation after DNBS-induced colitis. *PeerJ.* 2020;8:e8442. doi:10.7717/peerj.8442.
231. Shea-Donohue T, Urban JF. Neuroimmune modulation of gut function. *Handb Exp Pharmacol.* 2017;239:247-267. doi:10.1007/164\_2016\_109.
232. Gulbransen BD, Sharkey KA. Novel functional roles for enteric glia in the gastrointestinal tract. *Nat Rev Gastroenterol Hepatol.* 2012;9:625-632. doi:10.1038/nrgastro.2012.138.
233. Giaroni C, Zanetti E, Giuliani D, et al. Protein kinase c modulates NMDA receptors in the myenteric plexus of the guinea pig ileum during in vitro ischemia and reperfusion. *Neurogastroenterol Motil.* 2011;23:e91-e103. doi:10.1111/j.1365-2982.2010.01644.x.
234. Caputi V, Marsilio I, Filpa V, et al. Antibiotic-induced dysbiosis of the microbiota impairs gut neuromuscular function in juvenile mice. *Br J Pharmacol.* 2017;174:3623-3639. doi:10.1111/bph.13965.
235. Barajon I, Serrao G, Arnaboldi F, et al. Toll-like receptors 3, 4, and 7 are expressed in the enteric nervous system and dorsal root ganglia. *J Histochem Cytochem.* 2009;57:1013-1023. doi:10.1369/jhc.2009.953539.
236. Caputi V, Marsilio I, Cerantola S, et al. Toll-like receptor 4 modulates small intestine neuromuscular function through nitrenergic and purinergic pathways. *Front Pharmacol.* 2017;8:350. doi:10.3389/fphar.2017.00350.
237. Brun P, Giron MC, Qesari M, et al. Toll-like receptor 2 regulates intestinal inflammation by controlling integrity of the enteric nervous system. *Gastroenterology.* 2013;145:1323-1333. doi:10.1053/j.gastro.2013.08.047.
238. De Vadder F, Grasset E, Holm LM, et al. Gut microbiota regulates maturation of the adult enteric nervous system via enteric serotonin networks. *Proc Natl Acad Sci U S A.* 2018;115:6458-6463. doi:10.1073/pnas.1720017115.
239. Mawe GM, Hoffman JM. Serotonin signaling in the gastrointestinal tract. *Nat Rev Gastroenterol Hepatol.* 2013;10:473-486. doi:10.1038/nrgastro.2013.105.
240. Takaki M, Mawe GM, Barasch JM, Gershon MD, Gershon MD. Physiological responses of guinea-pig myenteric neurons secondary to the release of endogenous serotonin by tryptamine. *Neuroscience.* 1985;16:223-240. doi:10.1016/0306-4522(85)90059-4.
241. Forsythe P, Kunze WA. Voices from within: gut microbes and the CNS. *Cell Mol Life Sci.* 2013;70:55-69. doi:10.1007/s00018-012-1028-z.
242. Mcvey Neufeld KA, Mao YK, Bienenstock J, Foster JA, Kunze WA. The microbiome is essential for normal gut intrinsic primary afferent neuron excitability in the mouse. *Neurogastroenterol Motil.* 2013;25:183-e88. doi:10.1111/nmo.12049.
243. Husebye E, Hellström PM, Sundler F, Chen J, Midtvedt T. Influence of microbial species on small intestinal myoelectric activity and transit in germ-free rats. *Am J Physiol Gastrointest Liver Physiol.* 2001;280:G368-G380. doi:10.1152/ajpgi.2001.280.3.g368.
244. Garrett WS. Cancer and the microbiota. *Science (New York, NY).* 2015;348:80-86. doi:10.1126/science.aaa4972.
245. Trompette A, Gollwitzer ES, Yadava K, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med.* 2014;20:159-166. doi:10.1038/nm.3444.
246. Sundin J, Aziz I, Nordlander S, et al. Evidence of altered mucosa-associated and fecal microbiota composition in patients with Irritable Bowel Syndrome. *Sci Rep.* 2020;10:593. doi:10.1038/s41598-020-57468-y.
247. Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. *Cell Microbiol.* 2014;16:1024-1033. doi:10.1111/cmi.12308.
248. Zhuang X, Xiong L, Li L, Li M, Chen M. Alterations of gut microbiota in patients with irritable bowel syndrome: a systematic review and meta-analysis. *J Gastroenterol Hepatol (Australia).* 2017;32:28-38. doi:10.1111/jgh.13471.
249. Smoller J. The genetics of stress-related disorders: PTSD, depression, and anxiety disorders. *Neuropsychopharmacology.* 2016;41:297-319. doi:10.1038/npp.2015.266.
250. Laskaratos F-M, Goodkin O, Thoua NM, Murray CD. Irritable bowel syndrome. *Medicine (United Kingdom).* 2015;43:266-270. doi:10.1016/j.mpmed.2015.02.010.

251. Kennedy PJ, Cryan JF, Dinan TG, Clarke G. Irritable bowel syndrome: a microbiome-gut-brain axis disorder? *World J Gastroenterol.* 2014;20:14105-14125. doi:10.3748/wjg.v20.i39.14105.
252. Jeffery IB, O'Toole PW, Öhman L, et al. An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. *Gut.* 2012;61:997-1006. doi:10.1136/gutjnl-2011-301501.
253. Krogus-Kurikka L, Lyra A, Malinen E, et al. Microbial community analysis reveals high level phylogenetic alterations in the overall gastrointestinal microbiota of diarrhoea-predominant irritable bowel syndrome sufferers. *BMC Gastroenterol.* 2009;9:95. doi:10.1186/1471-230X-9-95.
254. Bercik P, Verdu EF, Collins SM. Is irritable bowel syndrome a low-grade inflammatory bowel disease? *Gastroenterol Clin North Am.* 2005;34:235-245. doi:10.1016/j.gtc.2005.02.007.
255. Belmonte L, Beutheu Youmba S, Bertiaux-Vandaële N, et al. Role of toll like receptors in irritable bowel syndrome: differential mucosal immune activation according to the disease subtype. *PLoS ONE.* 2012;7:e42777. doi:10.1371/journal.pone.0042777.
256. Koçak E, Akbal E, Köklü S, Ergül B, Can M. The colonic tissue levels of TLR2, TLR4 and nitric oxide in patients with irritable bowel syndrome. *Intern Med.* 2016;55:1043-1048. doi:10.2169/internalmedicine.55.5716.
257. Cussotto S, Sandhu KV, Dinan TG, Cryan JF. The neuroendocrinology of the microbiota-gut-brain axis: a behavioural perspective. *Front Neuroendocrinol.* 2018;51:80-101. doi:10.1016/j.yfrne.2018.04.002.
258. Foley S, Garsed K, Singh G, et al. Impaired uptake of serotonin by platelets from patients with irritable bowel syndrome correlates with duodenal immune activation. *Gastroenterology.* 2011;140:1434-1443.e1. doi:10.1053/j.gastro.2011.01.052.
259. Kerckhoffs APM, ter Linde JJM, Akkermans LMA, Samsom M. SERT and TPH-1 mRNA expression are reduced in irritable bowel syndrome patients regardless of visceral sensitivity state in large intestine. *Am J Physiol Gastrointest Liver Physiol.* 2012;302:G1053-G1060. doi:10.1152/ajpgi.00153.2011.
260. Manocha M, Khan WI. Serotonin and GI disorders: an update on clinical and experimental studies. *Clin Transl Gastroenterol.* 2012;3:e13. doi:10.1038/ctg.2012.8.
261. Mawe GM, Coates MD, Moses PL. Intestinal serotonin signalling in irritable bowel syndrome. *Aliment Pharmacol Ther.* 2006;23:1067-1076. doi:10.1111/j.1365-2036.2006.02858.x.
262. Wheatcroft J, Wakelin D, Smith A, Mahoney CR, Mawe G, Spiller R. Enterochromaffin cell hyperplasia and decreased serotonin transporter in a mouse model of postinfectious bowel dysfunction. *Neurogastroenterol Motil.* 2005;17:863-870. doi:10.1111/j.1365-2982.2005.00719.x.
263. Cremon C, Carini G, Wang B, et al. Intestinal serotonin release, sensory neuron activation, and abdominal pain in irritable bowel syndrome. *Am J Gastroenterol.* 2011;106:1290-1298. doi:10.1038/ajg.2011.86.
264. Camilleri M, Boeckxstaens G. Dietary and pharmacological treatment of abdominal pain in IBS. *Gut.* 2017;66:966-974. doi:10.1136/gutjnl-2016-313425.
265. Zheng Y, Yu T, Tang Y, et al. Efficacy and safety of 5-hydroxytryptamine 3 receptor antagonists in irritable bowel syndrome: a systematic review and meta-analysis of randomized controlled trials. *PLoS ONE.* 2017;12:e0172846. doi:10.1371/journal.pone.0172846.
266. Baj A, Bistoletti M, Bosi A, Moro E, Giaroni C, Crema F. Marine toxins and nociception: potential therapeutic use in the treatment of visceral pain associated with gastrointestinal disorders. *Toxins.* 2019;11:449. doi:10.3390/toxins11080449.
267. Saulnier DM, Riehle K, Mistretta T-A, et al. Gastrointestinal microbiome signatures of pediatric patients with irritable bowel syndrome. *Gastroenterology.* 2011;141:1782-1791. doi:10.1053/j.gastro.2011.06.072.
268. Desbonnet L, Clarke G, Traplin A, et al. Gut microbiota depletion from early adolescence in mice: implications for brain and behaviour. *Brain Behav Immun.* 2015;48:165-173. doi:10.1016/j.bbi.2015.04.004.
269. Uusijärvi A, Bergström A, Simrén M, et al. Use of antibiotics in infancy and childhood and risk of recurrent abdominal pain—a Swedish birth cohort study. *Neurogastroenterol Motil.* 2014;26:841-850. doi:10.1111/nmo.12340.
270. Perez-Burgos A, Wang L, Mcvey Neufeld K-A, et al. The TRPV1 channel in rodents is a major target for antinociceptive effect of the probiotic *Lactobacillus reuteri* DSM 17938. *J Physiol.* 2015;593:3943-3957. doi:10.1113/JP270229.
271. McKernan DP, Fitzgerald P, Dinan TG, Cryan JF. The probiotic *Bifidobacterium infantis* 35624 displays visceral antinociceptive effects in the rat. *Neurogastroenterol Motil.* 2010;22:1029-1035. doi:10.1111/j.1365-2982.2010.01520.x.
272. Aguilera M, Cerdà-Cuellar M, Martínez V. Antibiotic-induced dysbiosis alters host-bacterial interactions and leads to colonic sensory and motor changes in mice. *Gut Microbes.* 2015;6:10-23. doi:10.4161/19490976.2014.990790.
273. Verdú EF, Bercik P, Verma-Gandhu M, et al. Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. *Gut.* 2006;55:182-190. doi:10.1136/gut.2005.066100.
274. Crouzet L, Gaultier E, Del'Homme C, et al. The hypersensitivity to colonic distension of IBS patients can be transferred to rats through their fecal microbiota. *Neurogastroenterol Motil.* 2013;25:e272-e282. doi:10.1111/nmo.12103.
275. Donovan MH, Tecott LH. Serotonin and the regulation of mammalian energy balance. *Front Neurosci.* 2013;7:36. doi:10.3389/fnins.2013.00036.
276. Browning KN. Role of central vagal 5-HT3 receptors in gastrointestinal physiology and pathophysiology. *Front Neurosci.* 2015;9:413. doi:10.3389/fnins.2015.00413.
277. Shajib MS, Baranov A, Khan WI. Diverse effects of gut-derived serotonin in intestinal inflammation. *ACS Chem Neurosci.* 2017;8:920-931. doi:10.1021/acscchemneuro.6b00414.
278. Pusceddu MM, Gareau MG. Visceral pain: gut microbiota, a new hope? *J Biomed Sci.* 2018;25:73. doi:10.1186/s12929-018-0476-7.
279. Clarke G, McKernan DP, Gaszner G, Quigley EM, Cryan JF, Dinan TG. A distinct profile of tryptophan metabolism along the kynurenine pathway downstream of toll-like receptor activation in irritable bowel syndrome. *Front Pharmacol.* 2012;3:90. doi:10.3389/fphar.2012.00090.
280. Fitzgerald P, Cassidy Eugene M, Clarke G, et al. Tryptophan catabolism in females with irritable bowel syndrome: relationship to interferon-gamma, severity of symptoms and psychiatric co-morbidity. *Neurogastroenterol Motil.* 2008;20:1291-1297. doi:10.1111/j.1365-2982.2008.01195.x.
281. Filpa V, Moro E, Protasoni M, Crema F, Frigo G, Giaroni C. Role of glutamatergic neurotransmission in the enteric nervous system and brain-gut axis in health and disease. *Neuropharmacology.* 2016;111:14-33. doi:10.1016/j.neuropharm.2016.08.024.
282. McRoberts J, Coutinho S, Marvizon J, et al. Role of peripheral N-methyl-D-aspartate (NMDA) receptors in visceral nociception in rats. *Gastroenterology.* 2001;120:1737-1748.
283. Seybold V, Nerves S. The role of peptides in central sensitization. *Handb Exp Pharmacol.* 2009;194:451-491.
284. Onaga T. Tachykinin: recent developments and novel roles in health and disease. *Biomol Concepts.* 2014;5:225-243.
285. Raddant A, Russo A. Calcitonin gene-related peptide in migraine: intersection of peripheral inflammation and central modulation. *Expert Rev Mol Med.* 2011;13:e36.
286. Kolhekar R, Gebhart G. NMDA and quisqualate modulation of visceral nociception in the rat. *Brain Res.* 1994;651:215-226.
287. Kolhekar R, Gebhart G. Modulation of spinal visceral nociceptive transmission by NMDA receptor activation in the rat. *J Neurophysiol.* 1996;75:2344-2353.
288. Chen A, Chen Y, Tang Y, et al. Hippocampal AMPARs involve the central sensitization of rats with irritable bowel syndrome. *Brain Behav.* 2017;7:e00650. doi:10.1002/brb3.650.
289. Majláth Z, Török N, Toldi J, Vécsei L. Memantine and kynurenic acid: current neuropharmacological aspects. *Curr Neuropharmacol.* 2016;14:200-209. doi:10.2174/1570159x1466615113123221.
290. Nagy-Grócz G, Zádor F, Dvorácskó S, et al. Interactions between the kynurenine and the endocannabinoid system with special emphasis on migraine. *Int J Mol Sci.* 2017;18:1617. doi:10.3390/ijms18081617.
291. Csáti A, Edvinsson L, Vécsei L, et al. Kynurenic acid modulates experimentally induced inflammation in the trigeminal ganglion. *J Headache Pain.* 2015;16:99. doi:10.1186/s10194-015-0581-x.
292. Lee C, Doo E, Choi JM, et al. The increased level of depression and anxiety in irritable bowel syndrome patients compared with healthy controls: systematic review and meta-analysis. *J Neurogastroenterol Motil.* 2017;23:349-362. doi:10.5056/jnm16220.
293. Moloney RD, Johnson AC, O'Mahony SM, Dinan TG, Greenwood-Van Meerveld B, Cryan JF. Stress and the microbiota-gut-brain axis in visceral pain: relevance to irritable bowel syndrome. *CNS Neurosci Ther.* 2016;22:102-117. doi:10.1111/cns.12490.
294. Holzer P, Farzi A, Hassan AM, Zenz G, Jacan A, Reichmann F. Visceral inflammation and immune activation stress the brain. *Front Immunol.* 2017;8:1613. doi:10.3389/fimmu.2017.01613.
295. Zheng P, Zeng B, Zhou C, et al. Gut microbiome remodeling induces depressive-like behaviours through a pathway mediated by the host's metabolism. *Mol Psychiatry.* 2016;21:786-796. doi:10.1038/mp.2016.44.
296. Jiang H, Ling Z, Zhang Y, et al. Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav Immun.* 2015;48:186-194. doi:10.1016/j.bbi.2015.03.016.
297. Naseribafrouei A, Hestad K, Avershina E, et al. Correlation between the human fecal microbiota and depression. *Neurogastroenterol Motil.* 2014;26:1155-1162. doi:10.1111/nmo.12378.
298. Kelly JR, Borre Y, O'Brien C, et al. Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. *J Psychiatr Res.* 2016;82:109-118. doi:10.1016/j.jpsychires.2016.07.019.
299. Sudo N, Chida Y, Aiba Y, et al. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J Physiol.* 2004;558:263-275. doi:10.1113/jphysiol.2004.063388.
300. Gareau MG, Jury J, MacQueen G. Probiotic treatment of rat pups normalises corticosterone release and ameliorates colonic dysfunction induced by maternal separation. *Gut.* 2007;56:1522-1528. doi:10.1136/gut.2006.117176corr1.

301. Lafuse WP, Gearinger R, Fisher S, Nealer C, Mackos AR, Bailey MT. Exposure to a social stressor induces translocation of commensal lactobacilli to the spleen and priming of the innate immune system. *J Immunol.* 2017;198:2383-2393. doi:10.4049/jimmunol.1601269.
302. Lyte M, Vulchanova L, Brown DR. Stress at the intestinal surface: catecholamines and mucosa-bacteria interactions. *Cell Tissue Res.* 2011;343:23-32. doi:10.1007/s00441-010-1050-0.
303. Marin IA, Goertz JE, Ren T, et al. Microbiota alteration is associated with the development of stress-induced despair behaviour. *Sci Rep.* 2017;7:43859. doi:10.1038/srep43859.
304. Wichers MC, Koek GH, Robaey G, Verkerk R, Scharpé S, Maes M. IDO and interferon-alpha-induced depressive symptoms: a shift in hypothesis from tryptophan depletion to neurotoxicity. *Mol Psychiatry.* 2005;10:538-544. doi:10.1038/sj.mp.4001600.
305. Clarke G, Fitzgerald P, Cryan JF, Cassidy EM, Quigley EM, Dinan TG. Tryptophan degradation in irritable bowel syndrome: evidence of indoleamine 2,3-dioxygenase activation in a male cohort. *BMC Gastroenterol.* 2009;9:6. doi:10.1186/1471-230X-9-6.
306. Keszthelyi D, Troost FJ, Jonkers DM, Kruijmel JW, Leue C, Masclee AAM. Decreased levels of kynurenic acid in the intestinal mucosa of IBS patients: relation to serotonin and psychological state. *J Psychosom Res.* 2013;74:501-504. doi:10.1016/j.jpsychores.2013.01.008.
307. Miladinovic T, Nashed MG, Singh G. Overview of glutamatergic dysregulation in central pathologies. *Biomolecules.* 2015;5:3112-3141. doi:10.3390/biom5043112.
308. Chang C-H, Hsiao Y-H, Chen Y-W, Yu Y-J, Gean P-W. Social isolation-induced increase in NMDA receptors in the hippocampus exacerbates emotional dysregulation in mice. *Hippocampus.* 2015;25:474-485. doi:10.1002/hipo.22384.
309. Ghasemi M, Phillips C, Trillo L, De Miguel Z, Das D, Salehi A. The role of NMDA receptors in the pathophysiology and treatment of mood disorders. *Neurosci Biobehav Rev.* 2014;47:336-358. doi:10.1016/j.neubiorev.2014.08.017.
310. Cao B, Zhu J, Zuckerman H, Rosenblat J. Pharmacological interventions targeting anhedonia in patients with major depressive disorder: a systematic review. *Prog Neuropsychopharmacol Biol Psychiatry.* 2019;92:109-117. doi:10.1016/j.pnpbp.2019.01.002.
311. Zanos P, Piantadosi SC, Wu HQ, et al. The prodrug 4-chlorokynurenic acid causes ketamine-like antidepressant effects, but not side effects, by NMDA/glycineB-site inhibition. *J Pharmacol Exp Ther.* 2015;355:76-85. doi:10.1124/jpet.115.225664.
312. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet.* 2017;390:2769-2778. doi:10.1016/S0140-6736(17)32448-0.
313. Zuo T, Ng SC. The gut microbiota in the pathogenesis and therapeutics of inflammatory bowel disease. *Front Microbiol.* 2018;9:2247. doi:10.3389/fmicb.2018.02247.
314. Marchesi JR, Adams DH, Fava F, et al. The gut microbiota and host health: a new clinical frontier. *Gut.* 2016;65:330-339. doi:10.1136/gutjnl-2015-309990.
315. Barnich N, Denizot J, Darfeuille-Michaud A. E. Coli-mediated gut inflammation in genetically predisposed Crohn's disease patients. *Pathol Biol.* 2013;61:e65-e69. doi:10.1016/j.patbio.2010.01.004.
316. Frank DN, St. Amand A, Feldman R, Boedeker E, Harpaz N, Pace N. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U S A.* 2007;104:13780-13785. doi:10.1073/pnas.0706625104.
317. Walker AW, Sanderson JD, Churcher C, et al. High-throughput clone library analysis of the mucosa-associated microbiota reveals dysbiosis and differences between inflamed and non-inflamed regions of the intestine in inflammatory bowel disease. *BMC Microbiol.* 2011;11:7. doi:10.1186/1471-2180-11-7.
318. Hoshi N, Schenten D, Nish SA, et al. MyD88 signalling in colonic mononuclear phagocytes drives colitis in IL-10-deficient mice. *Nat Commun.* 2012;3:1120. doi:10.1038/ncomms2113.
319. Gasche C, Bakos S, Dejaco C, Tillinger W, Zakeri S, Reinisch W. IL-10 secretion and sensitivity in normal human intestine and inflammatory bowel disease. *J Clin Immunol.* 2000;20:362-370.
320. De Jager PL, Franchimont D, Waliszewska A, et al. The role of the Toll receptor pathway in susceptibility to inflammatory bowel diseases. *Genes Immun.* 2007;8:387-397. doi:10.1038/sj.gene.6364398.
321. Pierik M, Joossens S, Van Steen K, et al. Toll-like receptor-1, -2, and -6 polymorphisms influence disease extension in inflammatory bowel diseases. *Inflamm Bowel Dis.* 2006;12:1-8. doi:10.1097/01.MIB.0000195389.11645.ab.
322. Malvin NP, Seno H, Stappenbeck TS. Colonic epithelial response to injury requires Myd88 signaling in myeloid cells. *Mucosal Immunol.* 2012;5:194-206. doi:10.1038/mi.2011.65.
323. Lomax AE, Fernandez E, Sharkey KA. Plasticity of the enteric nervous system during intestinal inflammation. *Neurogastroenterol Motil.* 2005;17:4-15. doi:10.1111/j.1365-2982.2004.00607.x.
324. Brierley SM, Linden DR. Neuroplasticity and dysfunction after gastrointestinal inflammation. *Nat Rev Gastroenterol Hepatol.* 2014;11:611-627. doi:10.1038/nrgastro.2014.103.
325. Nikolaus S, Schulte B, Al-Massad N, et al. Increased tryptophan metabolism is associated with activity of inflammatory bowel diseases. *Gastroenterology.* 2017;153:1504-1516.e2. doi:10.1053/j.gastro.2017.08.028.
326. Hisamatsu T, Okamoto S, Hashimoto M, et al. Novel, objective, multivariate biomarkers composed of plasma amino acid profiles for the diagnosis and assessment of inflammatory bowel disease. *PLoS ONE.* 2012;7:e31131. doi:10.1371/journal.pone.0031131.
327. Gupta NK, Thaker AI, Kanuri N, et al. Serum analysis of tryptophan catabolism pathway: correlation with Crohn's disease activity. *Inflamm Bowel Dis.* 2012;18:1214-1220. doi:10.1002/ibd.21849.
328. Hashimoto T, Perlot T, Rehman A, et al. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature.* 2012;487:477-481. doi:10.1038/nature11228.
329. Jürgens B, Hainz U, Fuchs D, Felzmann T, Heitger A. Interferon- $\gamma$ -triggered indoleamine 2,3-dioxygenase competence in human monocyte-derived dendritic cells induces regulatory activity in allogeneic T cells. *Blood.* 2009;114:3235-3243. doi:10.1182/blood-2008-12-195073.
330. Yeung AWS, Terentis AC, King NJC, Thomas SR. Role of indoleamine 2,3-dioxygenase in health and disease. *Clin Sci.* 2015;129:601-672. doi:10.1042/CS20140392.
331. Brooks AK, Lawson MA, Smith RA, Janda TM, Kelley KW, McCusker RH. Interactions between inflammatory mediators and corticosteroids regulate transcription of genes within the Kynurenine Pathway in the mouse hippocampus. *J Neuroinflammation.* 2016;13:98. doi:10.1186/s12974-016-0563-1.
332. Takamatsu M, Hirata A, Ohtaki H, et al. IDO1 plays an immunosuppressive role in 2,4,6-trinitrobenzene sulfate-induced colitis in mice. *J Immunol.* 2013;191:3057-3064. doi:10.4049/jimmunol.1203306.
333. Christmas D, Badawy A, Hince D, Davies S. Increased serum free tryptophan in patients with diarrhea-predominant irritable bowel syndrome. *Nutr Res.* 2010;30:678-688. doi:10.1016/j.nutres.2010.09.009.
334. Sofia MA, Ciorba MA, Meckel K, et al. Tryptophan metabolism through the kynurenine pathway is associated with endoscopic inflammation in ulcerative colitis. *Inflamm Bowel Dis.* 2018;24:1471-1480. doi:10.1093/ibd/izy103.
335. Sas K, Szabó E, Vécsei L. Mitochondria, oxidative stress and the kynurenine system, with a focus on ageing and neuroprotection. *Molecules.* 2018;23:191. doi:10.3390/molecules23010191.
336. Abenavoli L, Scarpellini E, Colica C, et al. Gut microbiota and obesity: a role for probiotics. *Nutrients.* 2019;11:2690. doi:10.3390/nu11112690.
337. Kaszaki J, Érces D, Varga G, Szabó A, Vécsei L, Boros M. Kynurenines and intestinal neurotransmission: the role of N-methyl-D-aspartate receptors. *J Neural Transm.* 2012;119:211-223. doi:10.1007/s00702-011-0658-x.
338. Varga G, Érces D, Fazekas B, Fulop M. N-Methyl-D-aspartate receptor antagonism decreases motility and inflammatory activation in the early phase of acute experimental colitis in the rat. *Neurogastroenterol Motil.* 2010;22:217-225. doi:10.1111/j.1365-2982.2009.01390.x.
339. Gyires K, Tóth ÉV, Zádori SZ. Gut inflammation: current update on pathophysiology, molecular mechanism and pharmacological treatment modalities. *Curr Pharm Des.* 2014;20:1063-1081.
340. Érces D, Varga G, Fazekas B, Kovacs T, Tokes T. N-methyl-D-aspartate receptor antagonist therapy suppresses colon motility and inflammatory activation six days after the onset of experimental colitis in rats. *Eur J Pharmacol.* 2012;691:225-234. doi:10.1016/j.ejphar.2012.06.044.
341. Goettel JA, Gandhi R, Kenison JE, et al. AHR activation is protective against colitis driven by T cells in humanized mice. *Cell Rep.* 2016;17:1318-1329. doi:10.1016/j.celrep.2016.09.082.
342. Monteleone I, Rizzo A, Sarra M, et al. Aryl hydrocarbon receptor-induced signals up-regulate IL-22 production and inhibit inflammation in the gastrointestinal tract. *Gastroenterology.* 2011;141:237-248. doi:10.1053/j.gastro.2011.04.007.
343. Wang Q, Yang K, Han B, et al. Aryl hydrocarbon receptor inhibits inflammation in DSS-induced colitis via the MK2/p-MK2/TTP pathway. *Int J Mol Med.* 2018;41:868-876. doi:10.3892/ijmm.2017.3262.
344. Lamas B, Richard ML, Leducq V, et al. CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. *Nat Med.* 2016;22:598-605. doi:10.1038/nm.4102.
345. Alexeev EE, Lanis JM, Kao DJ, et al. Microbiota-derived indole metabolites promote human and murine intestinal homeostasis through regulation of interleukin-10 receptor. *Am J Pathol.* 2018;188:1183-1194. doi:10.1016/j.ajpath.2018.01.011.
346. Whitfield-Cargile CM, Cohen ND, Chapkin RS, et al. The microbiota-derived metabolite indole decreases mucosal inflammation and injury in a



- murine model of NSAID enteropathy. *Gut Microbes*. 2016;7:246-261. doi:10.1080/19490976.2016.1156827.
347. Takamura T, Harama D, Fukumoto S, et al. Lactobacillus bulgaricus OLL1181 activates the aryl hydrocarbon receptor pathway and inhibits colitis. *Immunol Cell Biol*. 2011;89:817-822. doi:10.1038/icb.2010.165.
348. Linden DR, Chen JX, Gershon MD, Sharkey KA, Mawe GM. Serotonin availability is increased in mucosa of guinea pigs with TNBS-induced colitis. *Am J Physiol Gastrointest Liver Physiol*. 2003;285:G207-G216. doi:10.1152/ajpgi.00488.2002.
349. Oshima SI, Fujimura M, Fujimiya M. Changes in number of serotonin-containing cells and serotonin levels in the intestinal mucosa of rats with colitis induced by dextran sodium sulfate. *Histochem Cell Biol*. 1999;112:257-263. doi:10.1007/s004180050445.
350. Ghia JE, Li N, Wang H, et al. Serotonin has a key role in pathogenesis of experimental colitis. *Gastroenterology*. 2009;137:1649-1660. doi:10.1053/j.gastro.2009.08.041.
351. Bischoff SC, Mailer R, Pabst O, et al. Role of serotonin in intestinal inflammation: knockout of serotonin reuptake transporter exacerbates 2,4,6-trinitrobenzene sulfonic acid colitis in mice. *Am J Physiol Gastrointest Liver Physiol*. 2009;296:G685-G695. doi:10.1152/ajpgi.90685.2008.
352. Haub S, Ritze Y, Bergheim I, Pabst O, Gershon MD, Bischoff SC. Enhancement of intestinal inflammation in mice lacking interleukin 10 by deletion of the serotonin reuptake transporter. *Neurogastroenterol Motil*. 2010;22:826-834. doi:10.1111/j.1365-2982.2010.01479.x.
353. Kwon YH, Wang H, Denou E, et al. Modulation of gut microbiota composition by serotonin signaling influences intestinal immune response and susceptibility to colitis. *Cell Mol Gastroenterol Hepatol*. 2019;7:709-728. doi:10.1016/j.jcmgh.2019.01.004.
354. Narula N, Pinto-Sanchez MI, Calo NC, et al. Anxiety but not depression predicts poor outcomes in inflammatory bowel disease. *Inflamm Bowel Dis*. 2019;25:1255-1261. doi:10.1093/ibd/izy385.
355. Emge JR, Huynh K, Miller EN, et al. Modulation of the microbiota-gut-brain axis by probiotics in a murine model of inflammatory bowel disease. *Am J Physiol Gastrointest Liver Physiol*. 2016;310:G989-G998. doi:10.1152/ajpgi.00086.2016.
356. Bercik P, Park AJ, Sinclair D, et al. The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut-brain communication. *Neurogastroenterol Motil*. 2011;23:1132-1139. doi:10.1111/j.1365-2982.2011.01796.x.
357. Bercik P, Verdu EF, Foster JA, et al. Chronic gastrointestinal inflammation induces anxiety-like behaviour and alters central nervous system biochemistry in mice. *Gastroenterology*. 2010;139:2102-2112.e1. doi:10.1053/j.gastro.2010.06.063.
358. Savitz J, Dantzer R, Meier TB, et al. Activation of the kynurenine pathway is associated with striatal volume in major depressive disorder. *Psychoneuroendocrinology*. 2015;62:54-58. doi:10.1016/j.psyneuen.2015.07.609.