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Focus on the essentials: tryptophan metabolism and the microbiome-gut-brain axis

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The gut-brain axis is a bidirectional communication system between the central nervous system and the gastrointestinal tract, in which serotonin (5-HT) functions as a key neurotransmitter. Recent research has increasingly concentrated on tryptophan, the precursor to 5-HT and on the microbial regulation of tryptophan metabolism, with an emphasis on host-microbe control over kynurenine pathway metabolism and microbial-specific pathways that generate bioactive tryptophan metabolites. Here, we critically assess recent progress made towards a mechanistic understanding of the microbial regulation of tryptophan metabolism and microbiota-gut-brain axis homeostasis highlighting the role tryptophan metabolism plays in preclinical and clinical neuroscience and in the challenge to improve our understanding of how perturbed tryptophan metabolism contributes to stress-related psychiatric disorders.

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Introduction

Host-microbe interactions are now more routinely considered in the context of brain function and behavior. Establishing the mechanistic basis for this fascinating dialogue between the gut microbiome and the gut-brain axis has proved more challenging. As the field transitions beyond compositional assessments of the gut microbiome, tryptophan has come under increasing scrutiny as a pivotal essential amino acid in the lexicon of host-microbial crosstalk. Recent advances in this field continue to demarcate the indirect means through which our gut microbes influence host metabolic pathways [1,2]. Direct microbial metabolism of tryptophan also yields microbial metabolites and candidate interkingdom signaling molecules acting as an interface between the host and its resident microorganisms with important physiological implications both in the gut and the brain [3,4]. Support continues to accumulate from studies of a broad swathe of gut-brain axis disorders that the metabolism of tryptophan is perturbed and associated with alterations in the composition or function of the gut microbiome [2]. In this review, we critically evaluate the recent advances in this area as we strive towards a mechanistic understanding of the microbial regulation of gut-brain axis homeostasis and the implications for neuroscience.

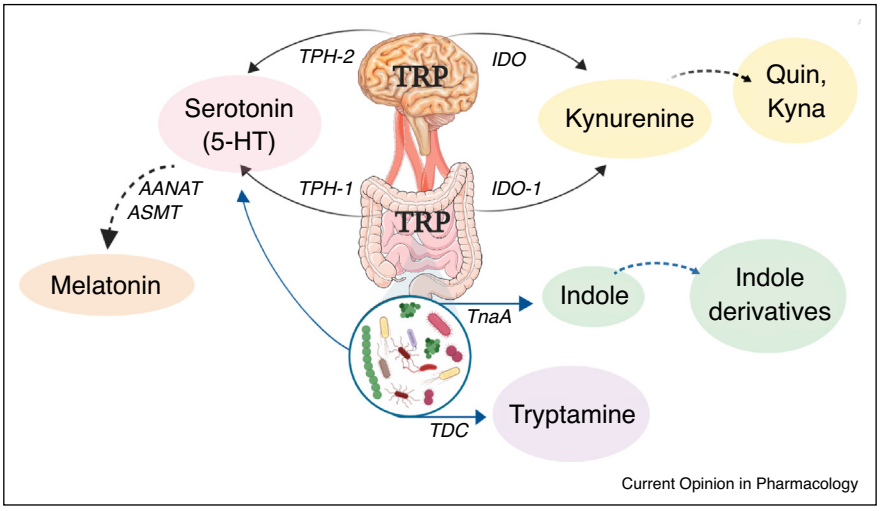
Tryptophan metabolism

Tryptophan metabolites have a huge impact both in the central nervous system (CNS) and in the periphery (see Figure 1). Once absorbed from the gut they become available in the circulation for distribution to target sites both peripherally and in the CNS. Tryptophan can also be metabolized directly by the gut microbiota and generate a range of indoles with diverse biological activity [5]. The combination of microbial and host gastrointestinal metabolism of tryptophan is thus likely an important factor in the systemic availability of tryptophan, as well as indoles, kynurenine and serotonin (5-HT) produced locally [3]. Much work has been done to better understand the impact of aberrant host tryptophan metabolism in psychiatric disorders and to identify the extent to which these molecular mechanisms dictate the impact of the gut microbiota on host physiology, brain function and behavior.

Tryptophan metabolism and gut-brain axis disorders

The importance of 5-HT in the gastrointestinal tract is consistent with the fact that ~95% of 5-HT is produced endogenously by enterochromaffin cells in the gut where it is involved in functions such as motility and secretion. Disruption in central and peripheral serotonergic signaling pathways are reported in GI disorders, such as inflammatory bowel disease (IBD) [6] and irritable bowel syndrome (IBS) – a functional

Figure 1



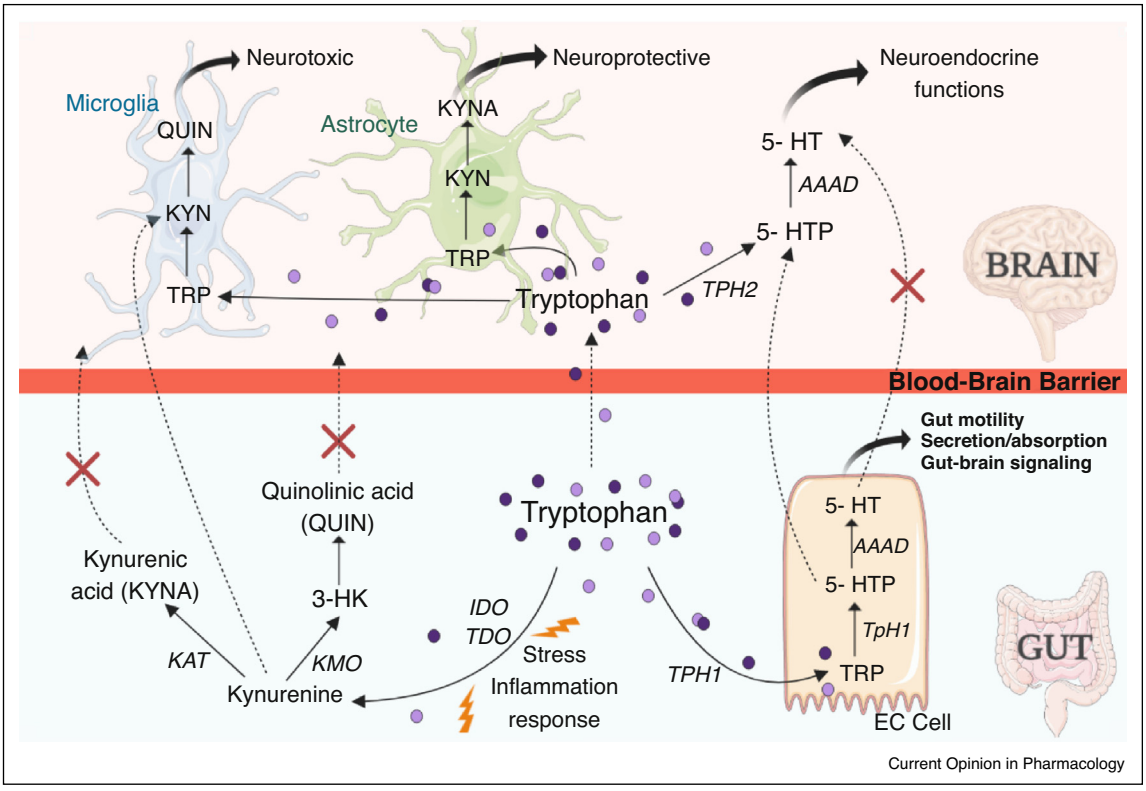
Tryptophan metabolism and the Microbiome-gut-brain axis.
An essential amino acid obtained from dietary proteins, tryptophan serves as a precursor to a variety of imperative bioactive molecules, some generated by the host and some fabricated by gut microbes (indicated by blue arrows). The most widely known fate of tryptophan is conversion into 5-HT and melatonin downstream. Nevertheless, a large majority of tryptophan is metabolized along the kynurenine pathway giving rise to molecules often collectively referred to as 'kynurenines'. The availability of tryptophan is also altered by gut microbes generating either indole and its derivatives, tryptamine or even 5-HT which can impact on gastrointestinal function via GPCR activation. An increasing number of studies highlight the importance of this pathway in metabolic and psychiatric disorders.

gastrointestinal disorder with significant psychiatric comorbidity – and in pathologies like autism spectrum disorder (ASD), a central nervous system disorder with comorbid gastrointestinal symptoms. For instance, activation of the kynurenine pathway has been reported in patients with IBD compared to controls. A sex-difference in tryptophan metabolism has been observed in both controls and patients with IBD characterised by lower serum tryptophan levels in female compared to males. The authors suggested this sex-difference could have important implications for understanding the increased female prevalence for some inflammatory phenotypes [7]. Differences in microbial subnetworks have been demonstrated in IBS patients compared to controls with respect to functional connectivity of brain regions in the somatosensory network and GI sensorimotor function, pointing to alterations in interactions within the brain-gut-microbiome axis [8]. The authors also pointed out the importance of members of the order *Clostridiales* in modulating host 5-HT biosynthesis and release. A recent study working with BTBR mice – a mouse-based model of ASD-like behaviors – exhibited microbiota-related impairments in 5-HT production in the intestine [9]. ASD is frequently associated with GI symptoms that can plausibly be linked to dysregulation of tryptophan metabolism in the gut [10,11,12]. In a study of children with autism, the authors demonstrated elevated concentrations in urine of xanthurenic acid and quinolinic acid suggesting preferential transformation from tryptophan at the expense of kynurenic acid [12].

The gut microbiome, stress related psychiatric disorders and tryptophan metabolism

An increasing number of studies report the impact of the intestinal microbiota on the fate and metabolism of tryptophan (see Figure 2). Importantly, the immuno-sensitive and stress-sensitive enzymes responsible for the initial conversion of L-tryptophan to L-kynurenine—indoleamine-2,3-deoxygenase (IDO) and tryptophan-2,3-dioxygenase (TDO) – may also be regulated directly or indirectly by the gut microbiome [14]. Indeed, experiments have revealed that germ-free (GF) mice (i.e. mice devoid of microrganisms, raised in a sterile environment) have reductions in kynurenine pathway metabolism that could be restored by colonization post-weaning [13]. Although the precise mechanisms are unclear, there are several potential routes through which the intestinal microbiota could regulate the expression and activity of kynurenine pathway enzymes. Examples include the production of hydrogen peroxide, microbial priming of the host immune system, activation of the aryl hydrocarbon receptor, the secretion of microbial metabolites influencing gut barrier integrity or via TLRs (see also Figure 3) [14]. Intestinal microbes are thought to be involved in stress-related disorders as exemplified by the study of Valles-Colomer *et al.* [15•] where differences in gut microbial composition were associated with lower quality of life (QoL) and depression status compared to healthy controls. In fact, certain strains of bacteria in the gut are able to directly utilize tryptophan consequently changing its availability to the host [16••,18•].

Figure 2



Tryptophan metabolism pathways and stress related Gut/Brain Interactions.
Aberrant tryptophan metabolism can occur in response to stress and inflammation in both the gut and the brain. An imbalance in the concentration of the different molecules that these pathways generates has consequences upon gut-brain signaling. In the gut, following immune activation or during the stress response, tryptophan is preferentially converted to kynurenine rather than 5-HT. Decreased 5-HT conversion from tryptophan – synthesized primarily by enterochromaffin cells – impacts on gastrointestinal motility and function. In the brain, tryptophan is metabolized along the kynurenine pathway by microglia and astrocytes leading to the formation of either kynurenic acid or quinolinic acid (by Astrocyte or Microglia, respectively). The majority of CNS kynurenine is derived from the periphery and once in the CNS, it can also participate in onwards metabolism. The balance between kynurenic acid and quinolinic acid is important for health and disease. Excessive activation of kynurenine metabolism may have neurotoxic consequences in clinical psychiatric and neurological disorders, such as depression.

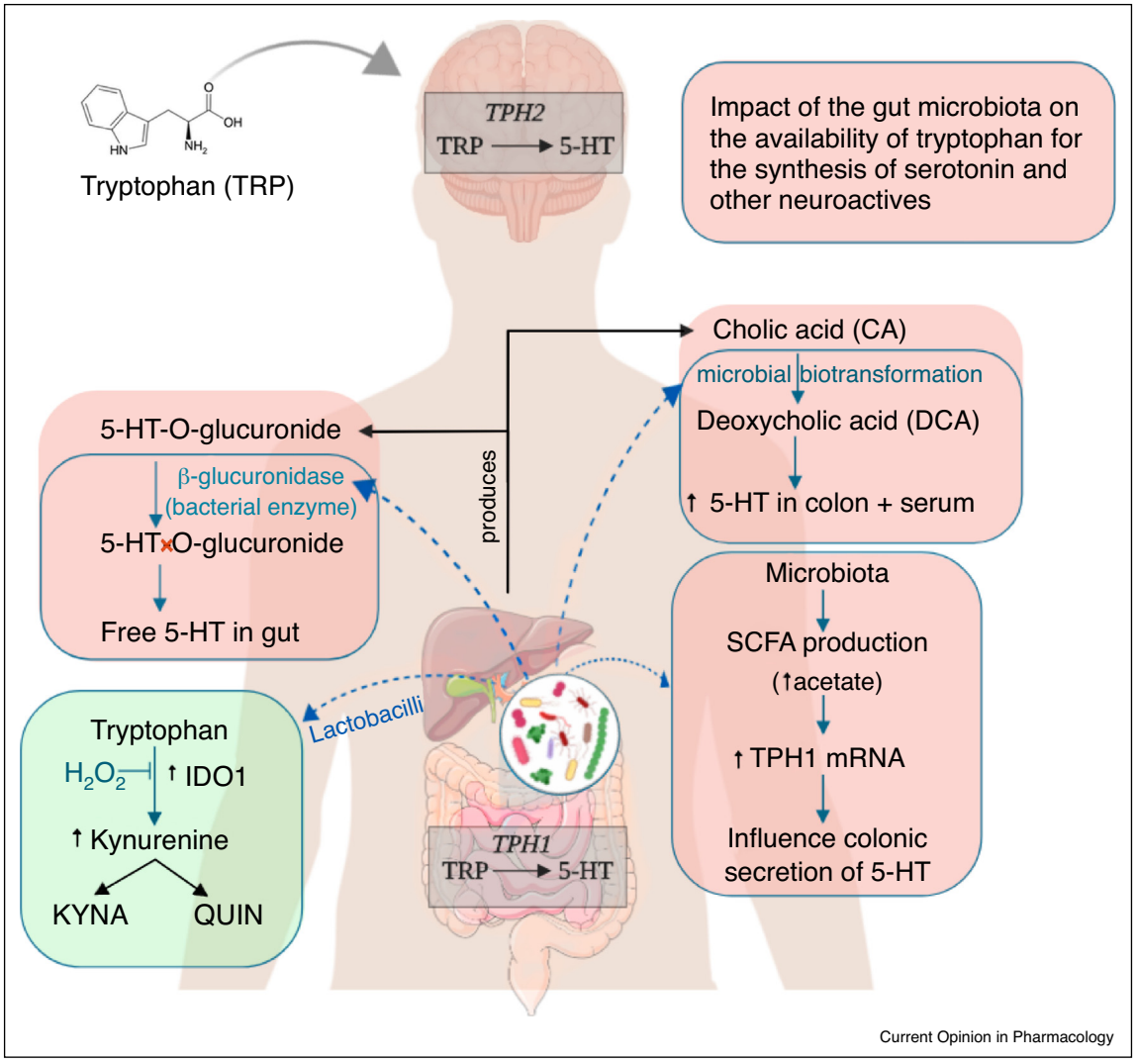
Kelly *et al.* [18•] performed fecal microbiota transplants from depressed patients to rats depleted of intestinal microbiota and demonstrated that the depressive phenotype is transmitted via the transfer of the intestinal microbiota as is the physiological hallmark of depression in terms of increased tryptophan metabolism along the kynurenine pathway.

Tryptophan and serotonin

Early studies in this field helped establish the principle that the gut microbiome regulated tryptophan availability and onward metabolism into 5-HT, not just locally in the gut but also in the CNS [2,13,14,20•,53•]. Acute tryptophan depletion (ATD) leads to increased depressive-like behavior and a stronger reduction of tryptophan, 5-HT and 5-hydroxyindoleacetic acid in the medial prefrontal cortex and hippocampus of GF than in SPF mice [21•]. Interestingly, following ATD, GF mice behave more similarly to SPF mice under basal conditions. The authors concluded that the serotonergic system of GF mice, which is abnormal at baseline, is more vulnerable to ATD. Administration of prebiotics (i.e. substrates that are selectively utilized by, and promotes the growth and/or activity of, beneficial host microorganisms compared to probiotics, which are live microorganisms consumed to produce a health benefit) in mice has shown antidepressant and anti-anxiolytic effects which underlines the possibility of exerting beneficial effects on the serotonergic system by therapeutic targeting of the gut microbiome, an appealing strategy, and one that can be expedited with an enhanced knowledge of the mechanisms underpinning this important emerging aspect of host-microbe dialogue [22].

Since 5-HT is mainly synthesized in the gut, it is not surprising that the intestinal microbiota can have a significant impact on its availability. Kwon *et al.* [23] demonstrated a considerable difference in the composition of the intestinal microbiota depending on whether or not the TPH-1 gene – the rate-limiting enzyme of 5-HT synthesis – was knocked

Figure 3



Candidate mechanisms for microbial regulation of tryptophan and serotonin release.
The availability and metabolism of tryptophan is under the influence of various intrinsic and extrinsic factors. There are a number of potential mechanisms through which the gut microbiome can influence tryptophan metabolism and the production of 5-HT or other metabolites. 5-HT can be synthesized both in the brain and in the gut by two different rate-limiting isoenzymes of tryptophan hydroxylase (TPH)—TPH1 and TPH2, respectively. It has been shown recently that some bacteria found in the gut microbiota, *in vitro*, are able to synthesize 5-HT. The microbiota has several ways to modulate 5-HT availability by regulating 5-HT secretion from enterochromaffin cells with short chain fatty acids (SCFA) increasing TPH1 mRNA expression. Microbial enzymes often biotransform metabolites produced by the liver, including the conversion of hepatic-derived cholic acid into deoxycholic acid leading to increased 5-HT in the colon and blood serum or by cleaving 5-HT-*O*-glucuronide, secreted into the gut lumen from the liver, into free 5-HT.

out and whether the progenitors were heterozygous or homozygous for this gene. They also provided evidence that 5-HT directly modulated the growth of commensal bacteria *in vitro* in a concentration-dependent and species-specific manner. It has also been demonstrated that mice with a TPH2 gene mutation, which leads to lower 5-HT biosynthesis in both enteric and CNS serotonergic neurons specifically, exhibit both brain and intestinal dysfunction and a slow release 5-HTP formulation was able to restore ENS-mediated GI function [24••].

Another proposed mechanism [16••] is by microbial biotransformation of cholic acid (CA) - secreted by the liver – into deoxycholic acid (DCA). Raising luminal concentrations of DCA in the colon of GF mice to levels seen in specific-pathogen free (SPF) mice sufficiently increases colon and serum 5-HT compared to vehicle-injected controls. This restoration of peripheral 5-HT correlates with elevations in colonic TPH1 expression. Sun *et al.* [25] further showed that the elevation of 5-HT seen in high fat diet rats can be restored to a conventional-like

level by use of fecal microbiota transplantation from control animals which may imply that elevated levels of DCA and CA could lead to the upregulation of TPH1 expression in the small intestine.

By colonization of previously GF mice with a complex microbiota, De Vadder *et al.* [26•] demonstrated that the gut microbiota stimulates neuronal and mucosal 5-HT release and that maturation of the adult ENS in GF mice requires 5-HT4R-specific signaling. Moreover, the microbiota likely affects 5-HT3 receptor expression to modulate colonic secretion [27]. This study also addressed the importance of short-chain fatty acids (SCFAs: microbial metabolites produced by bacterial fermentation of dietary fibers by the intestinal microbiota) in 5-HT colonic secretion and hypothesized that this effect could be mediated via acetate production by the gut microbiota. Another study confirmed this result *in vitro* by exposing BON cells (human EC cell model) to microbiota-derived SCFAs which significantly increased TPH1 mRNA expression [28]. There is thus growing evidence, including mechanistic insights, that the intestinal microbiota is a contributor to the colonic secretion of 5-HT. A very elegant study by Yano *et al.* [16••] introduced the concept that spore-forming bacteria could regulate host 5-HT synthesis in colonic enterochromaffin cells in mice. Indeed Mandić *et al.* [29] examined the specific involvement of the bacterium *Clostridium Ramosum* in colonic secretion of 5-HT in the gut and suggested it could be due to an induced expansion of enterochromaffin cells.

5-HT availability – in both the brain and intestine – depends on 5-HT transporter (SERT) function. Recently Singhal *et al.* [30] emphasized differences in cecal and fecal microbiota composition of $SERT^{-/-}$ or $SERT^{+/+}$ mice, concluding that SERT plays an important role in maintaining the homeostasis of the gut microbiota and that deficiency leads to loss of bacterial niches and altered microbial metabolic capabilities. In addition to being able to impact 5-HT production by acting on enterochromaffin cells, there are 5-HT-producing bacterial strains [14]. Lyte *et al.* [31] provided evidence that there could be a biogenic amine transport system in the biofilm of certain bacteria, particularly the genus *Lactobacillus*, demonstrating that bacteria are capable of modifying host availability of 5-HT. Since intestinal bacteria have an impact on 5-HT availability, bacteria may be indirectly involved in psychiatric diseases. It is also of considerable interest whether or not intestinal bacteria are reciprocally affected by the use of psychotropic drugs with serotonergic mechanisms of action. To address this question, Cussotto *et al.* [32] showed *in vitro* that two SSRI drugs, escitalopram and fluoxetine, modulate the growth of resident gut bacteria. The importance of these results were confirmed in a recent study which documented changes in microbial communities after chronic administration of fluoxetine [33].

One of the most understudied mechanisms by which the gut microbiota could influence the level of 5-HT in the gut lumen is deconjugation of 5-HT-*O*-glucuronide – produced by the liver – by β-glucuronidase, a bacterial enzyme. Ex-GF mice have the majority of 5-HT in an unconjugated form whereas GF mice have approximately 50% of the 5-HT in a conjugated form. This leads to the possibility that the intestinal microbiota could have a specific role in liberating biologically active free 5-HT [34]. Taken together, there is now evidence to postulate a role of microbiota in 5-HT production from EC cells and change in 5-HT availability in the host (see Figure 3).

Tryptophan and kynurenine

The available recent evidence suggests that the gut microbiota can exert an impact on important kynurenine pathway enzymes at multiple levels of the gut-brain axis. By comparing GF, colonized GF and conventionally colonized animals, Moloney *et al.* [35] highlighted the role of the intestinal microbiota in modulating the expression of miRNAs associated with the kynurenine pathway in the mouse hippocampus. Evidence shows that the microbial composition of chronically stressed mice changes compared to controls in a way that is associated with the development of depression-like behavior. A *Lactobacillus* strain, possibly by the production of H_2O_2 [36], exerted a protective role against stress-induced depression-like behavior associated with inhibition of intestinal IDO1 activity and a decreased circulating level of kynurenine. Another study focusing on kynurenine in MDD patients [37] supports those results as it showed that administration of *Lactobacillus plantarum* leads to a decrease in kynurenine concentrations and improved cognitive function. Kazemi *et al.* [38] also demonstrated the benefit of an eight-week probiotic treatment – *Lactobacillus helveticus* and *Bifidobacterium longum* supplementation – in MDD patients. This treatment resulted in a significant decrease in kynurenine/tryptophan ratio (used here as a marker of IDO activity) in serum samples compared to placebo. Furthermore, REGA3 has recently been identified [39] as an antimicrobial protein within the GI tract able to affect the composition of the gut microbiota towards an increase in *Lactobacillus*. Interestingly, REG3A-associated increases in *Lactobacillus* promotes production of kynurenine in gut epithelial cells in mice. By working with mice with an IDO-1 gene knockout in the context of obesity, Laurans *et al.* [40] found an increase of IL-22 target genes such as the antimicrobial proteins (Reg3g and Reg3b) in the intestine of High Fat Diet fed Ido1 −/− mice compared to high fat diet wild type mice highlighting that obesity may be associated with a microbiota-associated shift in tryptophan metabolism towards kynurenine production. Since the flow of kynurenine across the blood brain barrier is considered critical to its role in CNS pathology, harnessing the gut microbiota as a control point for kynurenine generation could have important therapeutic implications.

Tryptophan and melatonin

Melatonin is a hormone synthetized from 5-HT and mostly secreted by the pineal gland in mammals, but it can also act locally and be synthetized by several organs including the sites within the gastrointestinal tract. Its best known role is the regulation of the circadian rhythm but has also been shown to affect multiple molecular pathways including immune function, apoptosis, proliferation, angiogenesis and oxidative stress [41]. Sleep deprivation is a common burden that must be considered seriously as it can impact the autonomic nervous system, endocrine system and immune function, and that can be a trigger factor of metabolic or mental diseases. Gao *et al.* [42] investigated the effect of melatonin in a mouse model of sleep deprivation and found melatonin mediated sleep-deprivation induced mucosal injury and altered gut microbiota composition. Intriguingly, they show that sleep deprivation negatively impacts the diversity and richness of colonic microbiota and that melatonin supplementation greatly improves this state. In the context of weaning stress, melatonin supplementation was able to improve body weight gain and intestinal morphology and to increase the richness indices of intestinal microbiota and shape the composition of intestinal microbiota in conventionally colonized mice [43]. However, in both antibiotic-treated and GF mice, melatonin failed to affect intestinal morphology suggesting that there could be an involvement of intestinal microbiota in the regulatory functions of melatonin in intestinal physiology. Interestingly, a third study [44] focused on lipid metabolism found that melatonin supplementation in high fat diet fed mice alleviated the lipid accumulation and was able to reverse gut microbiota dysbiosis. They also showed results that suggest that melatonin can act on the intestinal microbiota by increasing the number of acetic acid-producing strains. These recent studies suggest that melatonin acts on the intestinal microbiota in several very different contexts, which implies that its role is essential in many physiological conditions and that its impact should not to be neglected in future studies.

Tryptophan and indole

Indole is produced from tryptophan via the enzyme tryptophanase by multiple indole-producing bacteria [45]. This metabolite plays a significant role for their survival and controls diverse physiological processes such as antimicrobial response, biofilm formation, motility and a range of other functions. Importantly, animal cells cannot synthesize indole. However, indole can be oxidized by non-indole-producing bacteria or eukaryotes into several biologically active derivatives [46]. Understanding how these tryptophan derivatives, synthesized by certain bacteria, can impact the host is an important research objective. There is growing evidence that these molecules have an impact at both peripheral and cerebral level, in particular through the binding to certain receptors such as the aryl hydrocarbon receptor (AHR) [4,47•], that promotes the expression of inflammation associated genes. Indole has been detected in the human gut at concentrations of 250–1100 μM [4] and indole derivatives synthetized by gut bacteria have been found in blood [19], peripheral tissues, urine and brain tissues which suggests an important role of those bacterial compounds. Some indole-derivatives are characterized by neurodepressive properties – namely oxindole and isatin – and excessive production of

Table 1

AHR ligands and their physiological impact on host metabolism

AHR ligands	Model	Potential physiological impact	Ref
Indole, 3-methyl indole, 2-oxindole	*In vitro* ligand binding, ligand structure activity analyses	Influence the transcription of important factors of the immune system.	[52]
Indoxyl-3-sulfate (I3S)	Mouse model of multiple sclerosis	Regulation of genes in neuroinflammation.	[53•]
Skatole/3-methylindole	Human colon cancer cell line Caco-2	Acts on the intestinal epithelial cells by AHR-dependent or AHR-independent activation pathways regulating the amount of IEC death.	[54]
Indole-3-propionic acid (IPA)	Model of high-fat diet mice	Might be reducing intestinal permeability and plasma LPS.	[55]
Indole-3-propionic acid (IPA)	Tryptophan-rich diet in rats	Contributes to changes in body weight gain.	[56]
Indole, skatole and indoleacetic acid	Humans	Influence of indoles derivatives on hedonic food intake and obesity by acting on the extended reward network.	[57]
Indoleacrylic acid (IA)	Model of colitis in mice	Indoleacrylic acid (IA) has a beneficial effect on intestinal epithelial barrier function and mitigates inflammatory responses by immune cells.	[58•]
AHR ligands	Fecal microbiota transplant (FMT) from CARD −/− mice into WT or GF mice	The FMT resulted in an increase sensitivity to colitis observed in mice depleted of CARD9 gene.	
	CARD9 is one of the IBD susceptibility genes	These alterations might be due to an impaired ability of the microbiota of CARD9 −/− mice to catabolize tryptophan into AHR ligands.	[59••]

indole by the gut microbiota may adversely affect behavior in rats [47•].

An increasing number of studies are focusing on the activation of the AHR as it has shown to have profound effects upon immunological status of the GI tract. However, the range of ligands responsible for AHR-activation within the gut continues to expand [48]. Recently, indole and some of these derivatives have been shown to be potent activators or stimulators of AHR and thus to influence the transcription of important factors of the immune system. This discovery is potentially of major importance because it highlights activation of the host's immune system through metabolites produced by the intestinal microbiota. Koper *et al.* [49] studied the kinetics of tryptophan-derived AHR-ligands by using a Simulator of the Human Intestinal Microbial Ecosystem (SHIME) and were able to simulate the ascending, transverse and descending colon from fresh human fecal sample. Some kynurenine derivatives showed a constant concentration through the colon, while other metabolites showed increased or decreased concentrations through different regions, although the implications of these region-specific variations are unclear. Table 1 lists some key indoles thought to activate the AHR and their potential physiological impact.

Tryptophan and tryptamine

Clostridium sporogenes and *Ruminococcus gnavus* are strains present in the gut microbiota capable of decarboxylating tryptophan to tryptamine through the specific enzyme tryptophan decarboxylase [50]. Tryptamine can play several roles in the gut such as signaling and has been shown to induce the release of 5-HT by enterochromaffin cells. Indeed, enteric neurons are able to take up tryptamine, which displaces 5-HT in intracellular synaptic vesicles, causing 5-HT release therefore might affect gastrointestinal motility [24••]. Bhattarai *et al.* [51••] uncovered a specific mechanism by which tryptamine-producing bacteria could accelerate gastrointestinal transit by activating the epithelial GPCR 5-HT4 receptor. To date, there are very few studies investigating the synthesis of tryptamine [50] by intestinal bacteria and the effect of this molecule in the body, but these promising initial studies indicate that there are some interesting discoveries to be made.

Concluding section

The essential role of tryptophan as a precursor to a range of bioactives important for signaling along the microbiome-gut-brain axis is increasingly appreciated in the context of psychological wellbeing and symptom generation. These tryptophan derivatives may facilitate both interorgan and interkingdom crosstalk and systemic availability of tryptophan, indoles, kynurenine, 5-HT and melatonin, each have an essential and unique aspect in maintaining signaling homeostasis along the microbiome-gut-brain axis. Microbial regulation of tryptophan availability and metabolism has important implications for many gut-brain axis disorders including GI disorders with psychiatric comorbidity, such as IBS, IBD and other CNS pathologies with GI dysfunction like ASD. These observations are likely missing pieces of the puzzle in understanding the origin and consequences of aberrant host tryptophan metabolism in many psychiatric disorders as well. Future research should seek to clarify the importance of lifestyle habits such as diet, sleep, daily activity and exercise for tryptophan metabolism in health and disease states. It remains to be seen if this fast accumulating information can be rationally integrated within a framework that enables mechanistically oriented therapeutic targeting of the gut microbiome.

Conflict of interest statement

APC Microbiome Ireland collaborates with a number of industry partners including Dupont Nutrition Biosciences APS, Cremo SA, Alkermes Inc., 4D Pharma PLC, Mead Johnson Nutrition, Nutricia Danone and Suntory Wellness. TGD has been an invited speaker at meetings organized by Servier, Lundbeck, Janssen and AstraZeneca and has received research funding from Mead Johnson, Cremo, Suntory Wellness, Nutricia and 4D Pharma. JFC has been an invited speaker at meetings organized by Mead Johnson, Yakult, Alkermes and Janssen and has received research funding from Mead Johnson, Cremo, Suntory Wellness, Nutricia, Dupont and 4D Pharma. GC has spoken at meetings sponsored by food and pharmaceutical companies including Janssen Ireland and Probi. This neither influenced nor constrained the content of this review.

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