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2 **Focus on the Essentials: Tryptophan Metabolism and the Microbiome-Gut-Brain Axis**

3  
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## ABSTRACT

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.

### Abbreviations:

5-HT: 5-hydroxytryptamine (serotonin)  
5-HTP: 5-hydroxytryptophan  
AAD: Aromatic amino Acid Decarboxylase  
AANAT: Arylalkylamine N-acetyltransferase  
AHR: Aryl Hydrocarbon receptor  
ASD: Autism Spectrum Disorder  
ASMT: Acetyl-Serotonin O-Methyltransferase  
CA: Cholic Acid  
CNS: Central Nervous System  
DCA: Deoxycholic Acid  
EC cells: Enterochromaffin cells  
ENS: Enteric Nervous System  
GF: Germ-Free  
GI tract: Gastrointestinal tract  
GPCR: G-Protein-Coupled Receptor  
IBD: Inflammatory Bowel Disease  
IBS: Irritable Bowel Syndrome  
IDO: Indoleamine dioxygenase  
KAT: Kynurenine aminotransferase  
KYNA: Kynurenic Acid  
L-KYN: L-Kynurenine  
MDD: Major Depressive Disorder  
QUIN: Quinolinic Acid  
SCFA: Short Chain Fatty Acid  
SERT: Serotonin Transporter  
SPF: Specific Pathogen-Free  
SSRI: Selective Serotonin Reuptake Inhibitors  
TDC: Tryptophan Decarboxylase  
TDO: Tryptophan 2,3-dioxygenase  
TnaA: Tryptophanase  
TPH: Tryptophan 5-hydroxylase

## 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 fig 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.

-- Insert Figure 1 here --

## TRYPTOPHAN METABOLISM & 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 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

120 authors also pointed out the importance of members of the order *Clostridiales* in modulating  
121 host 5-HT biosynthesis and release. A recent study working with BTBR mice – a mouse-based  
122 model of ASD-like behaviors – exhibited microbiota-related impairments in 5-HT production  
123 in the intestine [9]. ASD is frequently associated with GI symptoms that can plausibly be linked  
124 to dysregulation of tryptophan metabolism in the gut [11,12]. In a study of children with autism,  
125 the authors demonstrated elevated concentrations in urine of xanthurenic acid and quinolinic  
126 acid suggesting preferential transformation from tryptophan at the expense of kynurenic acid  
127 [12].

128

## 129 **THE GUT MICROBIOME, STRESS RELATED PSYCHIATRIC DISORDERS AND** 130 **TRYPTOPHAN METABOLISM**

131

132 An increasing number of studies report the impact of the intestinal microbiota on the fate and  
133 metabolism of tryptophan (see fig 2). Importantly, the immuno- and stress- sensitive enzymes  
134 responsible for the initial conversion of L-tryptophan to L-kynurenine – indoleamine-2,3-  
135 deoxygenase (IDO) and tryptophan-2,3-dioxygenase (TDO) – may also be regulated directly or  
136 indirectly by the gut microbiome. Indeed, experiments have revealed that germ-free (GF) mice  
137 (i.e. mice devoid of microorganisms, raised in a sterile environment) have reductions in  
138 kynurenine pathway metabolism that could be restored by colonization post-weaning [13].  
139 Although the precise mechanisms are unclear, there are several potential routes through which  
140 the intestinal microbiota could regulate the expression and activity of kynurenine pathway  
141 enzymes. Examples include the production of hydrogen peroxide, microbial priming of the host  
142 immune system, activation of the aryl hydrocarbon receptor, the secretion of microbial  
143 metabolites influencing gut barrier integrity or via TLRs (see also figure 3) [14]. Intestinal  
144 microbes are thought to be involved in stress-related disorders as exemplified by the study of  
145 Valles-Colomer et al. [15\*] where differences in gut microbial composition were associated  
146 with lower quality of life (QoL) and depression status compared to healthy controls. In fact,  
147 certain strains of bacteria in the gut are able to directly utilize tryptophan consequently changing  
148 its availability to the host [16\*\*,18]. Kelly et al. [18\*] performed fecal microbiota transplants  
149 from depressed patients to rats depleted of intestinal microbiota and demonstrated that the  
150 depressive phenotype is transmitted via the transfer of the intestinal microbiota as is the  
151 physiological hallmark of depression in terms of increased tryptophan metabolism along the  
152 kynurenine pathway.

153

154

-- Insert Figure 2 here --

155

156

## 156 **TRYPTOPHAN & SEROTONIN**

157

158 Early studies in this field helped establish the principle that the gut microbiome regulated  
159 tryptophan availability and onward metabolism into 5-HT, not just locally in the gut but also in  
160 the CNS [14,20,20\*]. Acute tryptophan depletion (ATD) leads to increased depressive-like  
161 behavior and a stronger reduction of tryptophan, 5-HT and 5-hydroxyindoleacetic acid in the  
162 medial prefrontal cortex and hippocampus of GF than in SPF mice [21\*]. Interestingly,  
163 following ATD, GF mice behave more similarly to SPF mice under basal conditions. The  
164 authors concluded that the serotonergic system of GF mice, which is abnormal at baseline, is  
165 more vulnerable to ATD. Administration of prebiotics (i.e. substrates that are selectively  
166 utilized by, and promotes the growth and/or activity of, beneficial host microorganisms  
167 compared to probiotics, which are live microorganisms consumed to produce a health benefit)  
168 in mice has shown antidepressant and anti-anxiolytic effects which underlines the possibility of  
169 exerting beneficial effects on the serotonergic system by therapeutic targeting of the gut

170 microbiome, an appealing strategy, and one that can be expedited with an enhanced knowledge  
171 of the mechanisms underpinning this important emerging aspect of host-microbe dialogue [22].  
172

173 Since 5-HT is mainly synthesized in the gut, it is not surprising that the intestinal microbiota  
174 can have a significant impact on its availability. Kwon et al. [23] demonstrated a considerable  
175 difference in the composition of the intestinal microbiota depending on whether or not the TPH-  
176 1 gene – the rate-limiting enzyme of 5-HT synthesis – was knocked out and whether the  
177 progenitors were heterozygous or homozygous for this gene. They also provided evidence that  
178 5-HT directly modulated the growth of commensal bacteria *in vitro* in a concentration-  
179 dependent and species-specific manner. It has also been demonstrated that mice with a TPH2  
180 gene mutation, which leads to lower 5-HT biosynthesis in both enteric and CNS serotonergic  
181 neurons specifically, exhibit both brain and intestinal dysfunction and a slow release 5-HTP  
182 formulation was able to restore ENS-mediated GI function [24\*\*].  
183

184 Another proposed mechanism [16\*\*] is by microbial biotransformation of cholic acid (CA) -  
185 secreted by the liver – into deoxycholic acid (DCA). Raising luminal concentrations of DCA in  
186 the colon of GF mice to levels seen in specific-pathogen free (SPF) mice sufficiently increases  
187 colon and serum 5-HT compared to vehicle-injected controls. This restoration of peripheral 5-  
188 HT correlates with elevations in colonic TPH1 expression. Sun et al. [25] further showed that  
189 the elevation of 5-HT seen in high fat diet rats can be restored to a conventional-like level by  
190 use of fecal microbiota transplantation from control animals which may imply that elevated  
191 levels of DCA and CA could lead to the upregulation of TPH1 expression in the small intestine.  
192

193 By colonization of previously GF mice with a complex microbiota, De Vadder et al. [26\*]  
194 demonstrated that the gut microbiota stimulates neuronal and mucosal 5-HT release and that  
195 maturation of the adult ENS in GF mice requires 5-HT<sub>4R</sub>-specific signaling. Moreover, the  
196 microbiota likely affects 5-HT<sub>3</sub> receptor expression to modulate colonic secretion [27]. This  
197 study also addressed the importance of short-chain fatty acids (SCFAs: microbial metabolites  
198 produced by bacterial fermentation of dietary fibers by the intestinal microbiota) in 5-HT  
199 colonic secretion and hypothesized that this effect could be mediated via acetate production by  
200 the gut microbiota. Another study confirmed this result *in vitro* by exposing BON cells (human  
201 EC cell model) to microbiota-derived SCFAs which significantly increased TPH1 mRNA  
202 expression [28]. There is thus growing evidence, including mechanistic insights, that the  
203 intestinal microbiota is a contributor to the colonic secretion of 5-HT. A very elegant study by  
204 Yano et al. [16\*\*] introduced the concept that spore-forming bacteria could regulate host 5-HT  
205 synthesis in colonic enterochromaffin cells in mice. Indeed Mandić et al. [29] examined the  
206 specific involvement of the bacterium *Clostridium Ramosum* in colonic secretion of 5-HT in  
207 the gut and suggested it could be due to an induced expansion of enterochromaffin cells.  
208

209 5-HT availability - in both the brain and intestine - depends on 5-HT transporter (SERT)  
210 function. Recently Singhal et al.[30] emphasized differences in cecal and fecal microbiota  
211 composition of SERT<sup>-/-</sup> or SERT<sup>+/+</sup> mice, concluding that SERT plays an important role in  
212 maintaining the homeostasis of the gut microbiota and that deficiency leads to loss of bacterial  
213 niches and altered microbial metabolic capabilities. In addition to being able to impact 5-HT  
214 production by acting on enterochromaffin cells, there are 5-HT-producing bacterial strains [14].  
215 Lyte et al.[31] provided evidence that there could be a biogenic amine transport system in the  
216 biofilm of certain bacteria, particularly the genus *Lactobacillus*, demonstrating that bacteria are  
217 capable of modifying host availability of 5-HT. Since intestinal bacteria have an impact on 5-  
218 HT availability, bacteria may be indirectly involved in psychiatric diseases. It is also of  
219 considerable interest whether or not intestinal bacteria are reciprocally affected by the use of

220 psychotropic drugs with serotonergic mechanisms of action. To address this question, Cussotto  
221 et al. [32] showed *in vitro* that two SSRI drugs, escitalopram and fluoxetine, modulate the  
222 growth of resident gut bacteria. The importance of these results were confirmed in a recent  
223 study which documented changes in microbial communities after chronic administration of  
224 fluoxetine [33].

225  
226 One of the most understudied mechanisms by which the gut microbiota could influence the  
227 level of 5-HT in the gut lumen is deconjugation of 5-HT-O-glucuronide - produced by the liver  
228 - by  $\beta$ -glucuronidase, a bacterial enzyme. Ex-GF mice have the majority of 5-HT in an  
229 unconjugated form whereas GF mice have approximately 50% of the 5-HT in a conjugated  
230 form. This leads to the possibility that the intestinal microbiota could have a specific role in  
231 liberating biologically active free 5-HT [34]. Taken together, there is now evidence to postulate  
232 a role of microbiota in 5-HT production from EC cells and change in 5-HT availability in the  
233 host (see fig 3).

234  
235 -- Insert Figure 3 here --

### 236 237 **TRYPTOPHAN & KYNURENINE**

238  
239 The available recent evidence suggests that the gut microbiota can exert an impact on important  
240 kynurenine pathway enzymes at multiple levels of the gut-brain axis. By comparing GF,  
241 colonized GF and conventionally colonized animals, Moloney et al. [35] highlighted the role  
242 of the intestinal microbiota in modulating the expression of miRNAs associated with the  
243 kynurenine pathway in the mouse hippocampus. Evidence shows that the microbial  
244 composition of chronically stressed mice changes compared to controls in a way that is  
245 associated with the development of depression-like behavior. A *Lactobacillus* strain, possibly  
246 by the production of H<sub>2</sub>O<sub>2</sub> [36], exerted a protective role against stress-induced depression-like  
247 behavior associated with inhibition of intestinal IDO1 activity and a decreased circulating level  
248 of kynurenine. Another study focusing on kynurenine in MDD patients [37] supports those  
249 results as it showed that administration of *Lactobacillus plantarum* leads to a decrease in  
250 kynurenine concentrations and improved cognitive function. Kazemi et al. [38] also  
251 demonstrated the benefit of an 8-week probiotic treatment - *Lactobacillus helveticus* and  
252 *Bifidobacterium longum* supplementation - in MDD patients. This treatment resulted in a  
253 significant decrease in kynurenine/tryptophan ratio (used here as a marker of IDO activity) in  
254 serum samples compared to placebo. Furthermore, REGA3 has recently been identified [39] as  
255 an antimicrobial protein within the GI tract able to affect the composition of the gut microbiota  
256 towards an increase in *Lactobacillus*. Interestingly, REG3A-associated increases in  
257 *Lactobacillus* promotes production of kynurenine in gut epithelial cells in mice. By working  
258 with mice with an IDO-1 gene knockout in the context of obesity, Laurans et al.[40] found an  
259 increase of IL-22 target genes such as the antimicrobial proteins (Reg3g and Reg3b) in the  
260 intestine of High Fat Diet fed *Ido1* *-/-* mice compared to high fat diet wild type mice  
261 highlighting that obesity may be associated with a microbiota-associated shift in tryptophan  
262 metabolism towards kynurenine production. Since the flow of kynurenine across the blood  
263 brain barrier is considered critical to its role in CNS pathology, harnessing the gut microbiota  
264 as a control point for kynurenine generation could have important therapeutic implications.

### 265 266 **TRYPTOPHAN & MELATONIN**

267  
268 Melatonin is a hormone synthesized from 5-HT and mostly secreted by the pineal gland in  
269 mammals, but it can also act locally and be synthesized by several organs including the sites

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

## 291 292 **TRYPTOPHAN & INDOLE**

293  
294 Indole is produced from tryptophan via the enzyme tryptophanase by multiple indole-producing  
295 bacteria [45]. This metabolite plays a significant role for their survival and controls diverse  
296 physiological processes such as antimicrobial response, biofilm formation, motility and a range  
297 of other functions. Importantly, animal cells cannot synthesize indole. However, indole can be  
298 oxidized by non-indole-producing bacteria or eukaryotes into several biologically active  
299 derivatives [46]. Understanding how these tryptophan derivatives, synthesized by certain  
300 bacteria, can impact the host is an important research objective. There is growing evidence that  
301 these molecules have an impact at both peripheral and cerebral level, in particular through the  
302 binding to certain receptors such as the aryl hydrocarbon receptor (AHR) [4,47], that promotes  
303 the expression of inflammation associated genes. Indole has been detected in the human gut at  
304 concentrations of 250-1100  $\mu\text{M}$  [4] and indole derivatives synthesized by gut bacteria have been  
305 found in blood, peripheral tissues, urine and brain tissues which suggests an important role of  
306 those bacterial compounds. Some indole-derivatives are characterized by neurodepressive  
307 properties - namely oxindole and isatin - and excessive production of indole by the gut  
308 microbiota may adversely affect behavior in rats [47\*].

309  
310 An increasing number of studies are focusing on the activation of the AHR as it has shown to  
311 have profound effects upon immunological status of the GI tract. However, the range of ligands  
312 responsible for AHR-activation within the gut continues to expand [48]. Recently, indole and  
313 some of these derivatives have been shown to be potent activators or stimulators of AHR and  
314 thus to influence the transcription of important factors of the immune system. This discovery is  
315 potentially of major importance because it highlights activation of the host's immune system  
316 through metabolites produced by the intestinal microbiota. Koper et al.[49] studied the kinetics  
317 of tryptophan-derived AHR-ligands by using a Simulator of the Human Intestinal Microbial  
318 Ecosystem (SHIME) and were able to simulate the ascending, transverse and descending colon  
319 from fresh human fecal sample. Some kynurenine derivatives showed a constant concentration

320 through the colon, while other metabolites showed increased or decreased concentrations  
321 through different regions, although the implications of these region-specific variations are  
322 unclear. Table 1 lists some key indoles thought to activate the AHR and their potential  
323 physiological impact.

### 324 **TRYPTOPHAN & TRYPTAMINE**

325  
326  
327 *Clostridium sporogenes* and *Ruminococcus gnavus* are strains present in the gut microbiota  
328 capable of decarboxylating tryptophan to tryptamine through the specific enzyme tryptophan  
329 decarboxylase [50]. Tryptamine can play several roles in the gut such as signaling and has been  
330 shown to induce the release of 5-HT by enterochromaffin cells. Indeed, enteric neurons are able  
331 to take up tryptamine, which displaces 5-HT in intracellular synaptic vesicles, causing 5-HT  
332 release therefore might affect gastrointestinal motility[24\*\*]. Bhattarai et al. [51\*\*] uncovered  
333 a specific mechanism by which tryptamine-producing bacteria could accelerate gastrointestinal  
334 transit by activating the epithelial GPCR 5-HT4 receptor. To date, there are very few studies  
335 investigating the synthesis of tryptamine [50] by intestinal bacteria and the effect of this  
336 molecule in the body, but these promising initial studies indicate that there are some interesting  
337 discoveries to be made.

### 338 **CONCLUDING SECTION**

339  
340  
341 The essential role of tryptophan as a precursor to a range of bioactives important for signaling  
342 along the microbiome-gut-brain axis is increasingly appreciated in the context of psychological  
343 wellbeing and symptom generation. These tryptophan derivatives may facilitate both interorgan  
344 and interkingdom crosstalk and systemic availability of tryptophan, indoles, kynurenine, 5-HT  
345 and melatonin, each have an essential and unique aspect in maintaining signaling homeostasis  
346 along the microbiome-gut-brain axis. Microbial regulation of tryptophan availability and  
347 metabolism has important implications for many gut-brain axis disorders including GI disorders  
348 with psychiatric comorbidity, such as IBS, IBD and other CNS pathologies with GI dysfunction  
349 like ASD. These observations are likely missing pieces of the puzzle in understanding the origin  
350 and consequences of aberrant host tryptophan metabolism in many psychiatric disorders as  
351 well. Future research should look to clarify the importance of lifestyle choices such as diet,  
352 sleep, daily activity and exercise participation for tryptophan metabolism in health and disease.  
353 It remains to be seen if this fast accumulating information can be rationally integrated within a  
354 framework that enables mechanistically-oriented therapeutic targeting of the gut microbiome.

### 355 **CONFLICT OF INTEREST STATEMENT**

356  
357  
358 APC Microbiome Ireland collaborates with a number of industry partners including Dupont  
359 Nutrition Biosciences APS, Cremo SA, Alkermes Inc., 4D Pharma PLC, Mead Johnson  
360 Nutrition, Nutricia Danone and Suntory Wellness. TGD has been an invited speaker at meetings  
361 organized by Servier, Lundbeck, Janssen and AstraZeneca and has received research funding  
362 from Mead Johnson, Cremo, Suntory Wellness, Nutricia and 4D Pharma. JFC has been an  
363 invited speaker at meetings organized by Mead Johnson, Yakult, Alkermes and Janssen and has  
364 received research funding from Mead Johnson, Cremo, Suntory Wellness, Nutricia, Dupont and  
365 4D Pharma. GC has spoken at meetings sponsored by food and pharmaceutical companies  
366 including Janssen Ireland and Probi. This neither influenced nor constrained the content of this  
367 review.

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369

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377

#### **REFERENCES AND RECOMMENDED READING**

378

379 Papers of particular interest, published within the period of review, have been highlighted as:

380

\* of special interest

381

\*\* of outstanding interest

382

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660 **Figure Legends**

661

662 **Figure 1: Tryptophan metabolism and the Microbiome-gut-brain axis**

663

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

673

674 **Figure 2: Tryptophan metabolism pathways and stress related Gut / Brain Interactions**

675

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

689

690 **Figure 3: Candidate mechanisms for microbial regulation of tryptophan and serotonin**  
691 **release.**

692

693 The availability and metabolism of tryptophan is under the influence of various intrinsic and  
694 extrinsic factors. There are a number of potential mechanisms through which the gut  
695 microbiome can influence tryptophan metabolism and the production of 5-HT or other  
696 metabolites. 5-HT can be synthesized both in the brain and in the gut by two different rate-

697 limiting isoenzymes of tryptophan hydroxylase (TPH) - TPH1 and TPH2, respectively. It has  
698 been shown recently that some bacteria found in the gut microbiota, *in vitro*, are able to  
699 synthesize 5-HT. The microbiota has several ways to modulate 5-HT availability by regulating  
700 5-HT secretion from enterochromaffin cells with short chain fatty acids (SCFA) increasing  
701 TPH1 mRNA expression. Microbial enzymes often biotransform metabolites produced by the  
702 liver, including the conversion of hepatic-derived cholic acid into deoxycholic acid leading to  
703 increased 5-HT in the colon and blood serum or by cleaving 5-HT-O-glucuronide, secreted into  
704 the gut lumen from the liver, into free 5-HT.

705

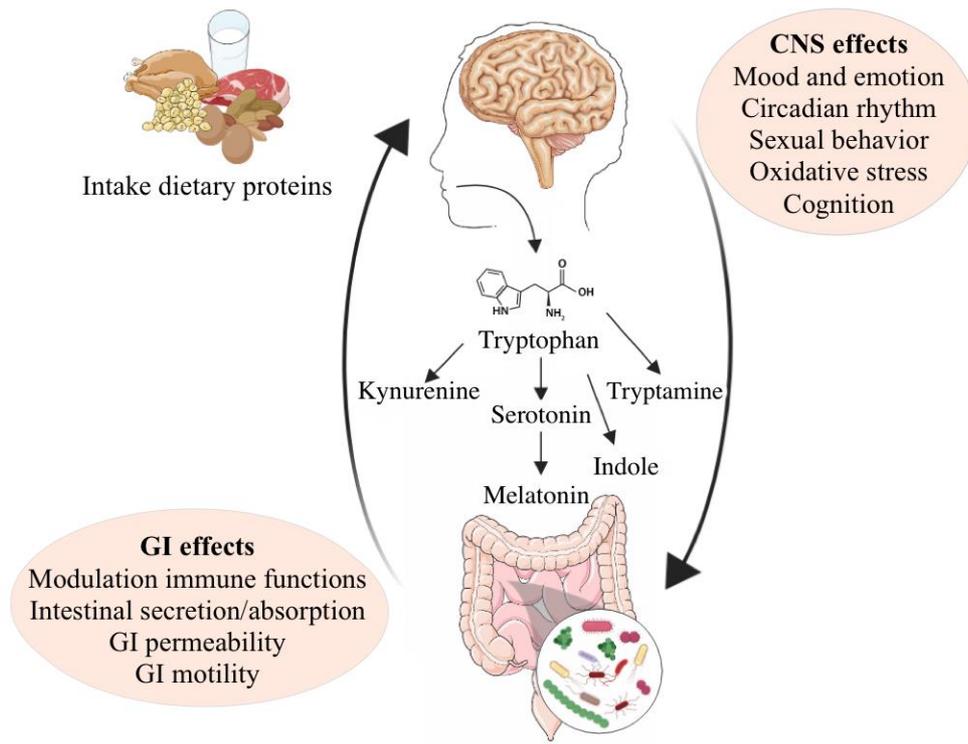
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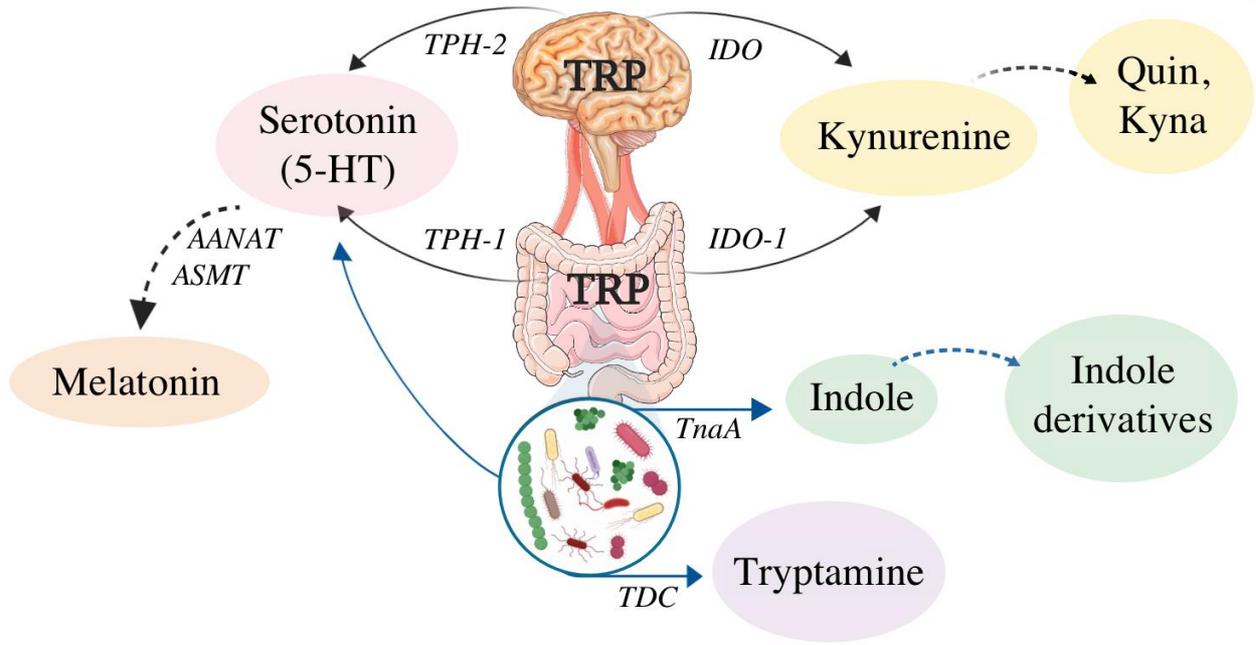
Table 1. AHR ligands and their physiological impact on host metabolism

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

709



**Graphical Abstract**



**Figure 1**

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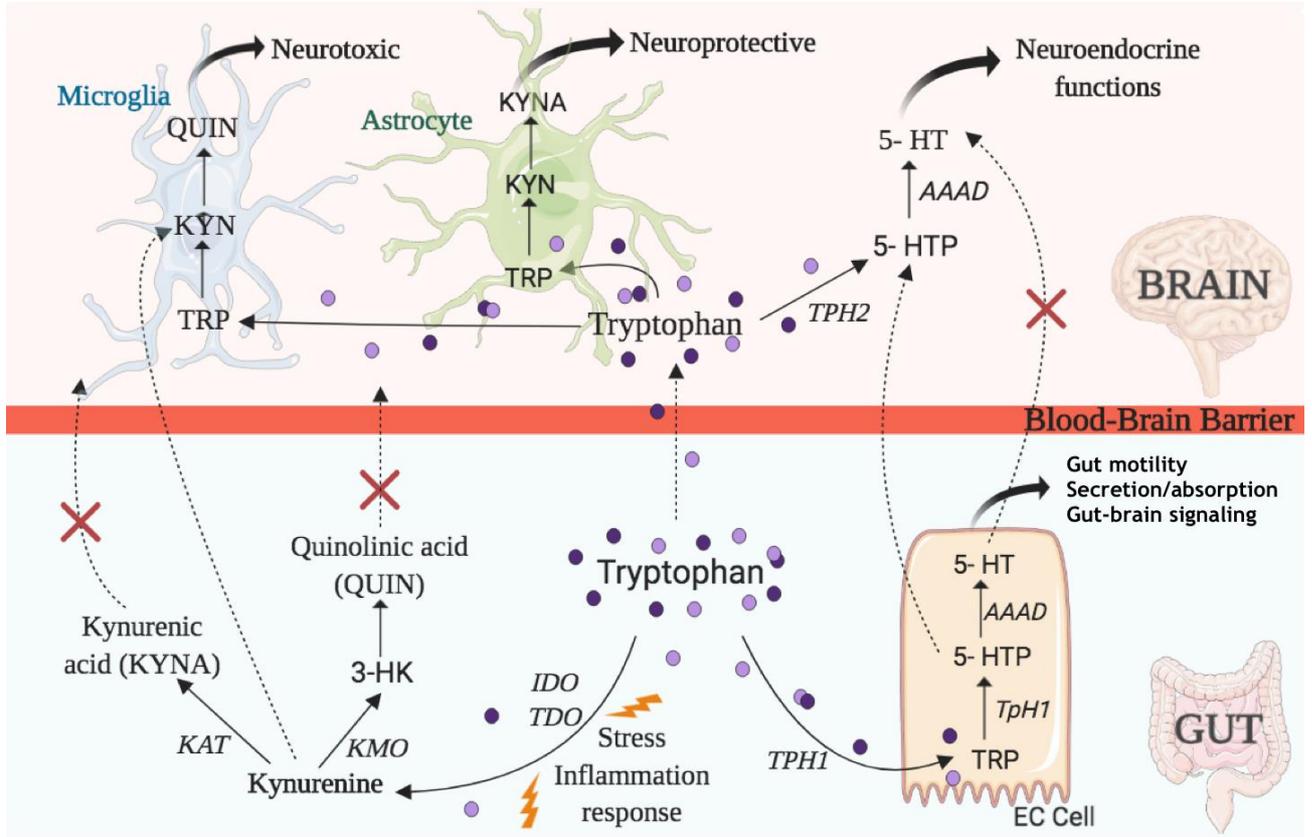
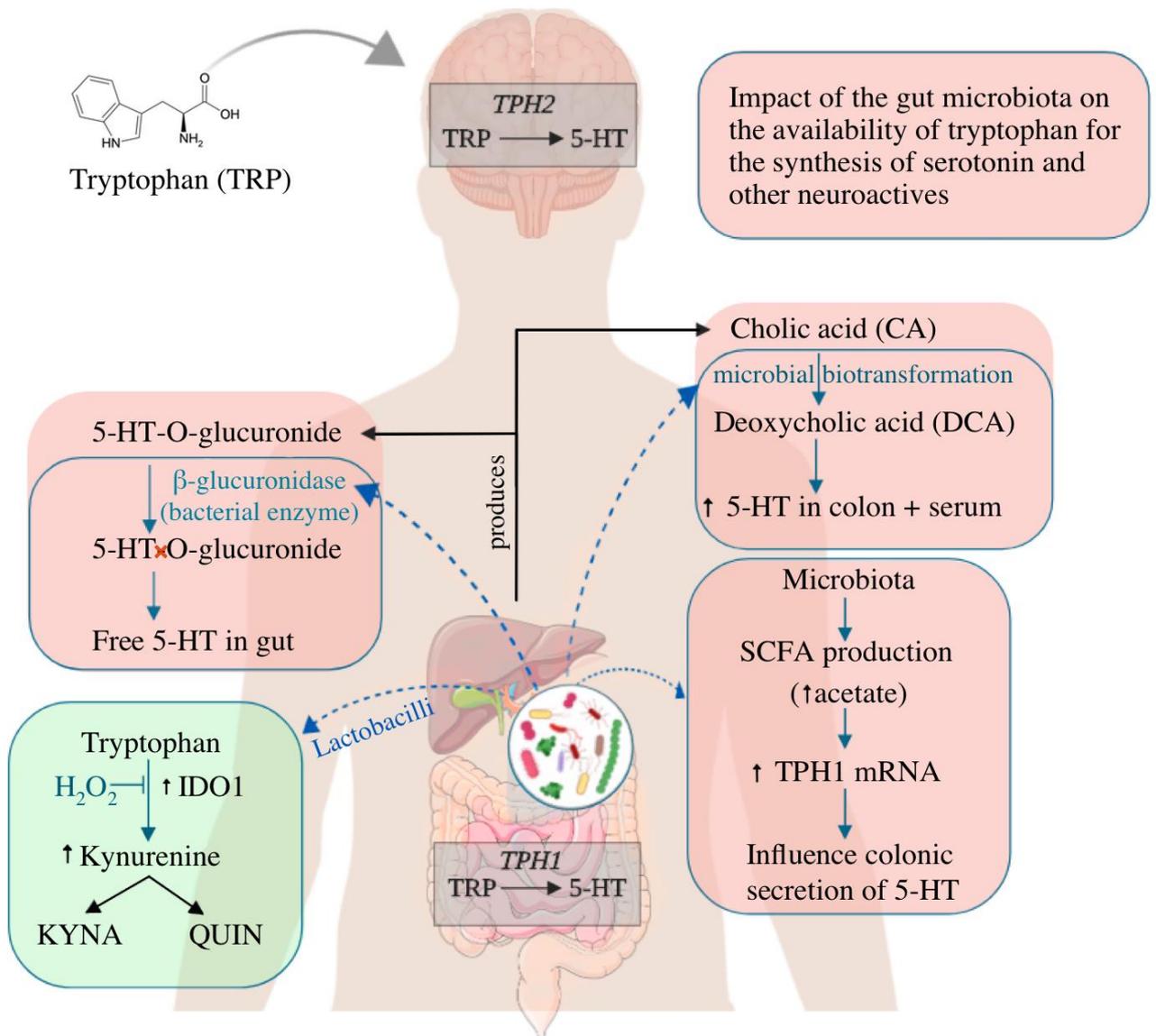


Figure 2

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**Figure 3**

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