Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Immunology

The double life of serotonin metabolites: in the mood for joining neuronal and immune systems

Giada Mondanelli and Claudia Volpi

Neuroimmune system is nowadays considered as one complex, but unique example of coordination between cellular and molecular networks, only apparently segregated, but strictly collaborating for the maintenance of body integrity. Too often, serotonin and its metabolites have been considered merely as neurotransmitters, when they have multiple effects spreading from the modulation of mood and behavioral processes to the regulation of a wide range of physiologic and pathophysiologic processes in most human organs, not least the immune response. The purpose of this review is to highlight the importance of metabolites generated along the serotonin pathway in the constant dialogue between neuroendocrine and immune systems; moreover, we would like to point out that the molecules produced in the two main routes of tryptophan metabolism are involved in a loop of self-regulation aimed at maintaining the equilibrium between these two metabolic pathways in the neuroimmune system, in both physiologic and pathologic conditions.

Address
Department of Medicine and Surgery, University of Perugia, Perugia, Italy

Corresponding author: Volpi, Claudia (claudia.volpi@unipg.it)

Current Opinion in Immunology 2021, **70**:1–6

This review comes from a themed issue on **Special section on indoles as immune regulators**

Edited by **Ursula Grohmann**

https://doi.org/10.1016/j.coi.2020.11.008

Introduction

Is inherent in human nature the need to pigeonhole and schematize the everevolving knowledge of the complex processes underlying the functioning of biological organisms. However, very frequently the boundary between two biological fields is thin, and the actors of one often have a leading role in the other as well. This is the case of neuroendocrine and immune systems. As our understanding of interactions between these two systems deepens, the once clear-cut distinction among the two is fading.

Today it is widely accepted that it is no longer possible to make a sharp distinction between the neuroendocrine system and the immune system, and that is more accurate to refer to them as neuroimmune system. A fascinating hypothesis assumes that in mammals these two systems may be evolutionary related through ancestral precursors, in order to guarantee tissue homeostasis and integrity [1]. In fact, neuroimmune interactions are found across multiple organs and have emerged as important regulators of physiology [2••]; however, functional interactions between the neuronal and the immune systems have also been described in several pathological conditions, including cancer, multiple sclerosis, chronic inflammatory disorders and autism [3]. The nervous and the immune system share many common features, both requiring coordinated actions of multiple cellular and molecular networks to sense, process and respond to endogenous and exogenous factors and to integrate complex environmental conditions. Moreover, either system can maintain a memory of earlier events, resulting in rapid responses to ever-evolving conditions. As additional examples of the connection and integration of these two systems, parasympathetic and sympathetic nerve fibers are implicated in the regulation of immune functions, as well as neurotransmitters and their receptors are expressed by immune cells, and neurons can direct immune pathways [4]. It has also been demonstrated that in the gut distinct neuronal and immune cells, sharing anatomical localization and functionally interacting, give rise to the so-called neuroimmune cell units (NICUs) [5]. The notion that connections between the gut, where many different immune cells reside, and the brain significantly contribute to wellness in humans led to the coining of the expression 'gut-brain axis'.

Role of serotonin pathway metabolites in neuroimmune communication

In addition to neurotransmitters, the mediators of the intense dialogue between nervous and immune systems are represented by a series of different molecules including cytokines, neuropeptides, chemokines and hormones [6,7].

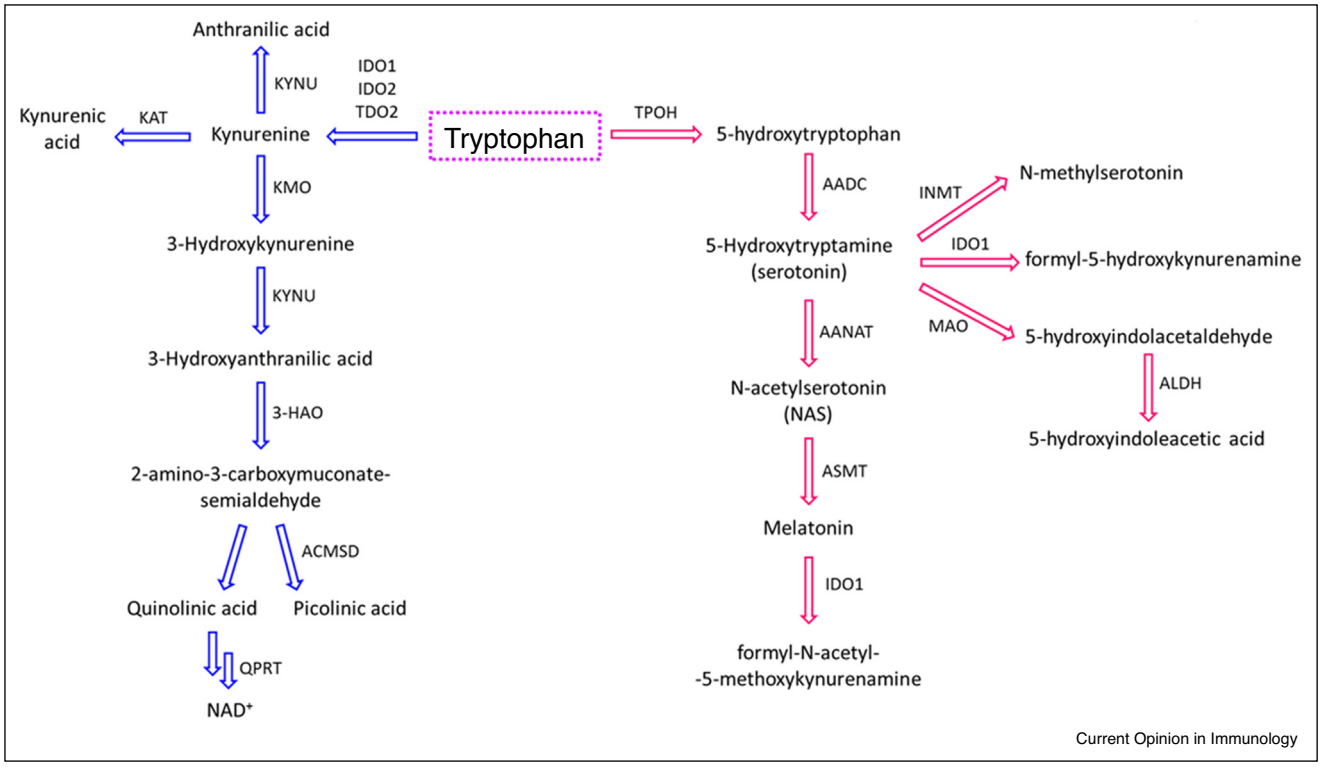
Among the molecules exerting a crucial role in connecting nervous and immune system, tryptophan and its metabolites are of paramount importance. L-tryptophan (Trp) is an essential amino acid that is obtained exclusively from dietary intake in humans. In addition to being a component for protein synthesis, Trp is also the substrate for the production of several bioactive substances, that have crucial roles in diverse physiological processes, ranging from cell growth to the coordination of organism's

responses to environmental and dietary signals, in which Trp metabolites serve as neurotransmitters and signaling molecules [8]. In mammals, it is estimated that the majority of free Trp is degraded through the kynurenine pathway (KP) and only 1% of Trp is used as a substrate for the biosynthesis of 5-hydroxytryptamine (5-HT or serotonin) and downstream metabolites (Figure 1). The prominent role of the metabolites generated along the kynurenine pathway both in the immune regulation and in the CNS is today consolidated [9•], but also serotonin and metabolites thereof are gaining a dominant position in the neuroimmune communication.

Serotonin has a strong reputation as a neurotransmitter; however, its role in the regulation of the immune responses is not far behind. Close to 95% of the serotonin in the body is actually synthesized from dietary Trp, stored and released by cells in the intestinal mucosa, known as enterochromaffin cells (EC cells). While the serotonin function as a mediator between brain and gut is well-established [10], much less is known about the connections between serotonin and the immune system. Many different immune cell types have proven to be able to possess the machinery to generate, store, respond to and/or transport serotonin, including T cells, macrophages and dendritic cells, both in the CNS and in the periphery. As a matter of the fact, drugs modulating serotonin reuptake (selective serotonin reuptake inhibitors, SSRIs) have been shown to possess immunosuppressive effects in various autoimmune diseases [11], and a strong relationship between inflammation, immune activation and neuropsychiatric disorders has also been demonstrated [12]. Moreover, evidences support an essential role of serotonin on immune cells in the pathogenesis of several autoimmune diseases [13].

An intriguing point to consider is the delicate balance between the Trp metabolites generated in the CNS along

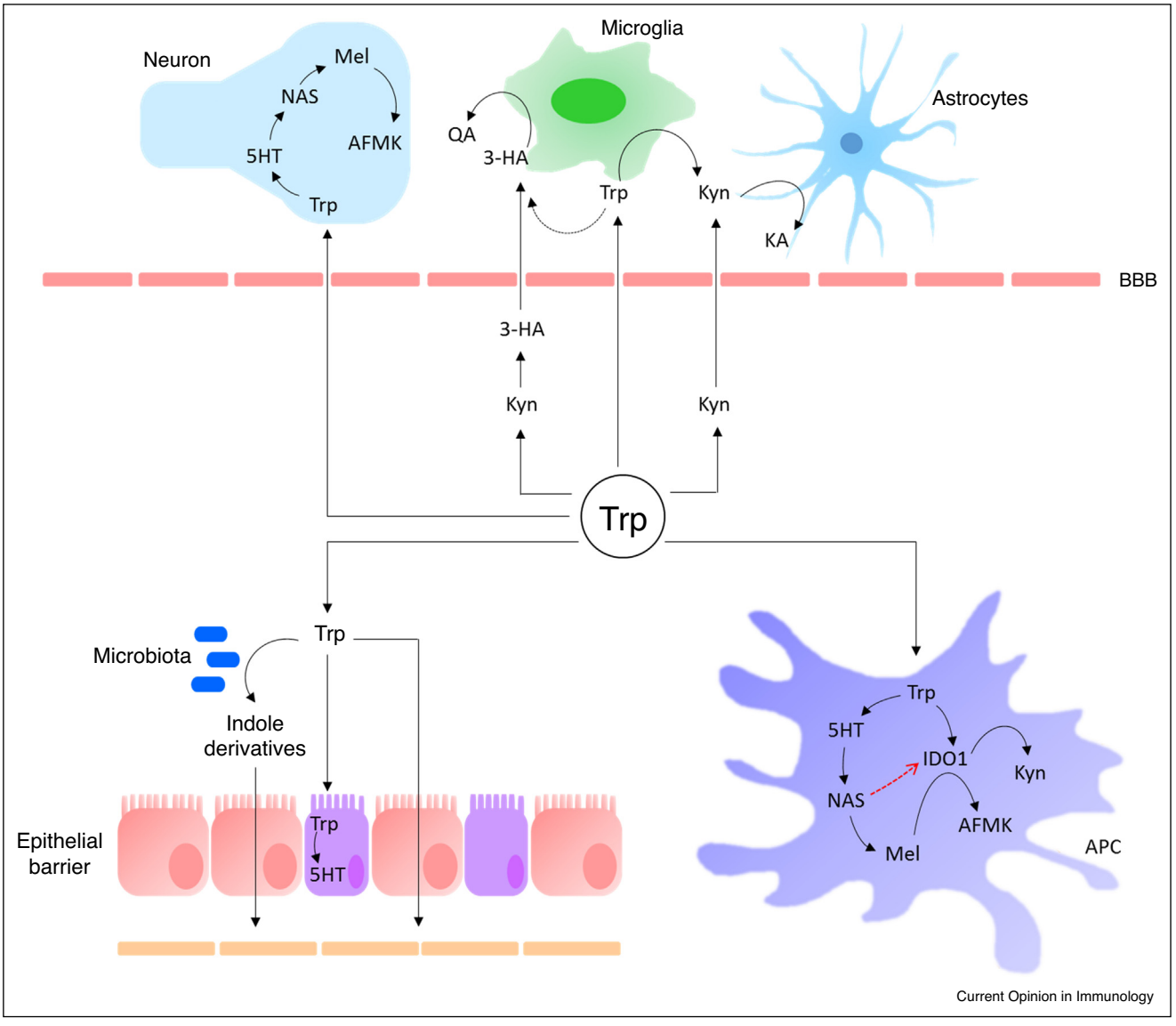
Figure 1



Tryptophan catabolism along the kynurenine and serotonin routes.
The majority of free L-tryptophan (Trp) is a substrate for the kynurenine pathway, in which the rate-limiting enzymes IDO1, IDO2 and TDO generates *N*-formylkynurenine that is rapidly metabolized by formamidase into L-kynurenine. Subsequently, L-kynurenine is converted by KATs, KYNU, and KMO into kynurenic acid, anthranilic acid, and 3-hydroxykynurenine, respectively. Trp can be degraded by TPOH into 5-hydroxytryptophan, which becomes substrate of AADC generating serotonin. Serotonin, in turn, is transformed by AANAT, INMT, IDO1 and MAO into N-acetylserotonin (NAS), *N*-methylserotonin, formyl-5-hydroxykynurenamine and 5-hydroxyindolacetaldehyde, respectively. NAS can be converted into melatonin, which then becomes the substrate of IDO1 to generate acetyl-*N*-formyl-5-methoxykynurenamine (AFMK).
Abbreviations: AADC Aromatic L-amino acid decarboxylase; AANAT Aralkylamine N-acetyltransferase ACMSD, aminocarboxymuconate semialdehyde decarboxylase; ASMT Acetylserotonin O-Methyltransferase; 3-HAO 3-hydroxyamino oxidase; IDO1 and IDO2 indoleamine 23-dioxygenases 1 and 2; INMT indolethylamine-*N*-methyltransferase KAT kynurenine aminotransferase; KMO kynurenine 3-monooxygenase; MAO Monoamine oxidases QPRT quinolinate phosphoribosyltransferase; TDO tryptophan 2,3-dioxygenase, TPOH, tryptophan-5-hydroxylase.

the kynurenine pathway and the serotonin ones (Figure 2). Decreased Trp influx to the brain (as a result of peripheral inflammation-induced and/or infection-induced degradation of Trp along the kynurenine pathway) and the subsequent reduction of serotonin and its metabolites in the CNS have been suggested to be underlying factors for mental illness and dysregulation of neuroendocrine axis [14]; in this way, enhanced IDO1 activity in the periphery may contribute to mood-lowering and neuronal degeneration when tryptophan availability in the CNS becomes low during periods of immune activation [15]. On the other hand, in neuroinflammatory conditions, the KP in the CNS is strongly up regulated, leading to the production of several neuroactive metabolites that can be either neuroprotective (such as kynurenic acid in astrocytes) or neurotoxic (quinolinic acid, mainly produced by microglial cells) or immunomodulatory. A contribution for the components of the KP has been tracked in various diseases of the CNS, such as

Figure 2



Tryptophan metabolism at the intersection of neuroendocrine- and immune roads.
L-tryptophan (Trp) and its metabolites (such as Kyn and 3-HA) are transported across the blood brain barrier (BBB) and taken up by neurons, microglia and astrocytes, which produce 5-HT (serotonin), quinolinic acid (QA) and kynurenic acid (KA), respectively. In the gut, Trp can be either metabolized by intestinal microbiota into indole derivatives or absorbed and released into the circulation. Alternatively, enterochromaffin cells transform Trp into 5-HT. In antigen presenting cells (APCs), both the serotonin and the kynurenine pathways are at stake. The enzyme indoleamine 2,3-dioxygenase 1 (IDO1) not only generates Kyn, but also acetyl-*N*-formyl-5-methoxykynurenamine (AFMK) along the serotonin route. *N*-acetylserotonin (NAS), that is, the intermediate of Melatonin (Mel) synthesis, activates IDO1 (dotted line) and thus fuels the production of more Kyn.

Alzheimer disease, amyotrophic lateral sclerosis, Huntington disease and multiple sclerosis [16]. Thus, IDO1 represents a crucial focal point around which neuroendocrine system and immune system functions are bond, affecting the availability of tryptophan and the shifting the balance toward the production of specific metabolites that give a voice to the complex neuroimmune dialogue.

Beside the effects of serotonin itself on the neuroimmune communication, whose mechanisms certainly deserve further investigations, serotonin also serves as a substrate for the synthesis of various downstream metabolites, some of them showing effects that spread both in the neuronal and in the immune system. Within the immune cells, four catabolic pathways for the degradation of serotonin have been described (Figure 1). One pathway leads to the generation of N-acetyl serotonin (NAS), which then acquires a methyl group to become melatonin. Subsequently, melatonin can be converted by the enzyme indoleamine 2,3-dioxygenase 1 (IDO1) to acetyl-*N*-formyl-5-methoxykynurenamine (AFMK). Serotonin itself can be a substrate of the enzyme IDO1 in a second breakdown pathway, generating formyl-5-hydroxykynurenamine. In a third pathway, serotonin is transformed into 5-hydroxyindoleacetic acid, while in a fourth catabolic pathway serotonin generates *N*-methylserotonin (Figure 1). Immune cells express all the machinery necessary for the breakdown of serotonin along all the pathways described above [17], suggesting a functional role for the metabolites generated from serotonin in the immune response.

NAS is a molecule endowed with multiple functions; very recently, an explanation of some of its neuroimmunemodulatory effects has been proposed by Mondanelli *et al.* [18•]. More in detail, NAS, in conventional dendritic cells, acts as allosteric modulator of IDO1, directly binding the enzyme in a specific allosteric binding-site and enhancing the production of kynurenine, which in turn functions as a ligand for the aryl hydrocarbon receptor (AhR). These findings prove the existence of an endogenous mechanism of cross-regulation of the two main metabolic routes of Trp metabolism (i.e. the kynurenine and the serotonin pathways). The specific effects of molecules, such as NAS, produced in the CNS and in the periphery by pathways active both in neuronal and immune cells reinforce the interconnection of the neuroendocrine-immune systems. Moreover, many other tryptophan byproducts, such as the kynurenine-derived metabolites kynurenic acid, cinnabarinic acid and xanthurenic acid, are endowed with the ability to bind AhR, but also indole compounds produced by intestinal microbiota, such as tryptamine, indole-3-acetic acid (IAA) and indole-3-aldehyde (IAld) are AhR confirmed ligands. All these molecules are well known for their dual activity as immune- and neuromodulators, constituting the aforementioned 'gut-brain axis', having a role in the regulation of neuroinflammatory processes as a linking bridge between the immune system and the CNS [19]. It clearly appears that not only tryptophan and its derivatives, but also AhR represents a central hub of integration not only between different metabolites and cell types, but also a crossroads of neuronal and immune systems.

The pleiotropic effects of NAS may not be its exclusive prerogative, since other serotonin metabolites could also exhibit similar behavior. Melatonin is classically associated with the regulation of circadian rhythms, following its release by the pineal gland; however, growing body of evidences show melatonin to be released by many cell types, including immune cells, and exerting immunomodulatory effects with implications in the pathogenesis of several autoimmune diseases [20]. Melatonin synthesis seems to be finely coordinated to such an extent that it's possible to speak of 'immune-pineal axis', in which a pivotal role is exerted by the transcription factor NF-κB, responsible for driving the switch of melatonin production from pinealocytes to macrophages/microglia in response to acute inflammatory responses, and back to pinealocytes upon inflammation resolution [21]. A sustained activation of immune-pineal axis, due to unresolved inflammatory processes, could be the basis of many neurological diseases, including Alzheimer's and Parkinson's disease [22], but also of cancer, in which the activation of immune-pineal axis participates in the complex association between the melatonergic system and cancer initiation/progression [21]. More intriguingly, as pointed out above, melatonin is also a substrate of IDO1, giving rise to AFMK, a COX-inhibitor capable of blocking the synthesis of prostaglandins, thus exerting anti-inflammatory and immunomodulatory effects [17]. The effects of the kynurenamines (such as AFMK or 5-hydroxykynurenamine) on the immune system are still not fully understood. A clue could derive by the traditional notion that 5-hydroxykynurenamine acts as a serotonin antagonist with subsequent effects in platelets aggregation [23], and mainly because IDO1 — the enzyme responsible for the synthesis of these molecules — is a key piece of the mechanisms leading to the regulation of the immune response.

Conclusions

When it comes to Trp metabolism in neuroendocrine-immune dialogue, metabolites along the kynurenine pathway take the lion's share, due to the plethora of evidences underlying their numerous effects in connecting the nervous and the immune system. Not only the metabolites generated by Trp catabolism in immune cells residing in the CNS and in the periphery, but also the ones derived by microbiota contribute to the maintenance of the neuroimmune bidirectional communication [2••]. Nevertheless in this scenario, where only a small amount of dietary Trp is intended to be converted to 5-HT, serotonin metabolites are emerging as key mediators of the neuroimmune system.

A main question that needs to be addressed is which mechanism underlies the Trp metabolism during the lifespan and the factors affecting the shift of the balance towards one destiny (kynurenine pathway) or the other (serotonin pathway). Indeed, the competition between 5-HT synthesis and kynurenine production in cells expressing both enzymatic pools (such as the immune and the neuronal cells) is under the control of various stimuli, including neurotransmitters, cytokines and hormones [24]. Moreover, environmental factors and variations in the health status can specifically modulate the activation of one pathway over the other. What is noteworthy is the relevance of IDO1 in the delicate balance between the two main metabolic fates of Trp: not only its activation leads to the production of several molecules (i.e. Kyn and metabolites thereof) involved in the maintenance of the body homeostasis, but also molecules generated along the serotonin pathway can function as a substrate (i.e. melatonin) or allosteric modulators (i.e. NAS) of IDO1, thus creating a circuit in which the two pathways are strictly interconnected. Even though, so far, there is no evidence of kynurenines acting as substrate or allosteric modulators of the enzymes involved in the serotonin pathway, it is possible to speculate that such a mechanism could be common to a relevant part of the Trp metabolites, in order to guarantee an appropriate equilibrium between the main metabolic routes of Trp consumption. These observations should be kept in mind when considering the relevance of Trp metabolites as neuroimmune communicators, due to their high potential to become biomarker and/or therapeutic targets in inflammatory, neurological and metabolic diseases, as well as in cancer, all pathologies in which a dense network of neuroimmune interactions have been well established.

Funding sources

This work was supported by the Italian Ministry of Education, University, and Research (PRIN 20173EAZ2Z to C. Volpi)

Conflict of interest statement

Nothing declared.

References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. Arendt D: **The evolution of cell types in animals: emerging principles from molecular studies**. *Nat Rev Genet* 2008, **9**:868-882 http://dx.doi.org/10.1038/nrg2416.

2. •• Huh JR, Veiga-Fernandes H: **Neuroimmune circuits in inter-organ communication**. *Nat Rev Immunol* 2020, **20**:217-228 http://dx.doi.org/10.1038/s41577-019-0247-z
This review summarize in a deep and fascinating way the discoveries about molecules, cells and functional units participating in neuroimmune communication.

3. Veiga-Fernandes H, Mucida D: **Neuro-immune interactions at barrier surfaces**. *Cell* 2016, **165**:801-811 http://dx.doi.org/10.1016/j.cell.2016.04.041.

4. Kioussis D, Pachnis V: **Immune and nervous systems: more than just a superficial similarity?** *Immunity* 2009, **31**:705-710 http://dx.doi.org/10.1016/j.immuni.2009.09.009.

5. Veiga-Fernandes H, Pachnis V: **Neuroimmune regulation during intestinal development and homeostasis**. *Nat Immunol* 2017, **18**:116-122 http://dx.doi.org/10.1038/ni.3634.

6. Maier SF: **Bi-directional immune-brain communication: Implications for understanding stress, pain, and cognition**. *Brain Behav Immun* 2003, **17**:69-85 http://dx.doi.org/10.1016/s0889-1591(03)00032-1.

7. Ordovas-Montanes J, Rakoff-Nahoum S, Huang S, Riol-Blanco L, Barreiro O, von Andrian UH: **The regulation of immunological processes by peripheral neurons in homeostasis and disease**. *Trends Immunol* 2015, **36**:578-604 http://dx.doi.org/10.1016/j.it.2015.08.007.

8. Cervenka I, Agudelo LZ, Ruas JL: **Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health**. *Science* 2017, **357** http://dx.doi.org/10.1126/science.aaf9794.

9. • Platten M, Nollen EAA, Röhrig UF, Fallarino F, Opitz CA: **Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond**. *Nat Rev Drug Discov* 2019, **18**:379-401 http://dx.doi.org/10.1038/s41573-019-0016-5
This review deepens the role of tryptophan metabolites generated along the kynurenine pathway in physiologic and pathologic settings.

10. Fung TC, Olson CA, Hsiao EY: **Interactions between the microbiota, immune and nervous systems in health and disease**. *Nat Neurosci* 2017, **20**:145-155 http://dx.doi.org/10.1038/nn.4476.

11. Wu H, Denna TH, Storkersen JN, Gerriets VA: **Beyond a neurotransmitter: the role of serotonin in inflammation and immunity**. *Pharmacol Res* 2019, **140**:100-114 http://dx.doi.org/10.1016/j.phrs.2018.06.015.

12. Höglund E, Øverli Ø, Winberg S: **Tryptophan Metabolic pathways and brain serotonergic activity: a comparative review**. *Front Endocrinol* 2019, **10**:158 http://dx.doi.org/10.3389/fendo.2019.00158.

13. Wan M, Ding L, Wang D, Han J, Gao P: **Serotonin: a potent immune cell modulator in autoimmune diseases**. *Front Immunol* 2020, **11**:186 http://dx.doi.org/10.3389/fimmu.2020.00186.

14. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW: **From inflammation to sickness and depression: when the immune system subjugates the brain**. *Nat Rev Neurosci* 2008, **9**:46-56 http://dx.doi.org/10.1038/nrn2297.

15. Gostner JM, Geisler S, Stonig M, Mair L, Sperner-Unterweger B, Fuchs D: **Tryptophan metabolism and related pathways in psychoneuroimmunology: the impact of nutrition and lifestyle**. *Neuropsychobiology* 2020, **79**:89-99 http://dx.doi.org/10.1159/000496293.

16. Huang Y-S, Ogbechi J, Clanchy FI, Williams RO, Stone TW: **IDO and kynurenine metabolites in peripheral and CNS disorders**. *Front Immunol* 2020, **11**:388 http://dx.doi.org/10.3389/fimmu.2020.00388.

17. Arreola R, Becerril-Villanueva E, Cruz-Fuentes C, Velasco-Velázquez MA, Garcés-Alvarez ME, Hurtado-Alvarado G, Quintero-Fabian S, Pavón L: **Immunomodulatory effects mediated by serotonin**. *J Immunol Res* 2015, **2015** http://dx.doi.org/10.1155/2015/354957 354957.

18. • Mondanelli G, Coletti A, Greco FA, Pallotta MT, Orabona C, Iacono A, Belladonna ML, Albini E, Panfili E, Fallarino F *et al.*: **Positive allosteric modulation of indoleamine 2,3-dioxygenase 1 restrains neuroinflammation**. *Proc Natl Acad Sci U S A* 2020, **117**:3848-3857 http://dx.doi.org/10.1073/pnas.1918215117
This article shows that a serotonin pathway metabolite, N-acetyl serotonin, acts as an allosteric modulator of the immuneregulating enzyme indoleamine 2,3-dioxygenase, linking serotonin pathway to the kynurenine one and contributing to the neuroimmune crosstalk in a model of experimental autoimmune encephalomyelitis.

19. Liu Y, Hou Y, Wang G, Zheng X, Hao H: **Gut microbial metabolites of aromatic amino acids as signals in host-microbe interplay**. *Trends Endocrinol Metab* 2020, **31**:818-834 http://dx.doi.org/10.1016/j.tem.2020.02.012.

20. Skarlis C, Anagnostouli M: **The role of melatonin in multiple sclerosis**. *Neurol Sci Off J Ital Neurol Soc Ital Soc Clin Neurophysiol* 2020, **41**:769-781 http://dx.doi.org/10.1007/s10072-019-04137-2.

21. Markus RP, Fernandes PA, Kinker GS, da Silveira Cruz-Machado S, Marçola M: **Immune-pineal axis - acute inflammatory responses coordinate melatonin synthesis by pinealocytes and phagocytes**. *Br J Pharmacol* 2018, **175**:3239-3250 http://dx.doi.org/10.1111/bph.14083.

22. Beriwal N, Namgyal T, Sangay P, Al Quraan AM: **Role of immune-pineal axis in neurodegenerative diseases, unraveling novel hybrid dark hormone therapies**. *Heliyon* 2019, **5**:e01190 http://dx.doi.org/10.1016/j.heliyon.2019.e01190.

23. Okuma M, Tokuyama T, Senoh S, Hirata F, Hayaishi O: **Antagonism of 5-hydroxykynurenamine against serotonin action on platelet aggregation**. *Proc Natl Acad Sci U S A* 1976, **73**:643-645 http://dx.doi.org/10.1073/pnas.73.2.643.

24. Palego L, Betti L, Rossi A, Giannaccini G: **Tryptophan Biochemistry: structural, nutritional, metabolic, and medical aspects in humans**. *J Amino Acids* 2016, **2016** http://dx.doi.org/10.1155/2016/8952520 8952520.