Toward manipulating serotonin signaling via the microbiota–gut–brain axis
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It is now well established in humans that there is a bidirectional pathway of communication between the central and enteric nervous systems in which members of the microbiome participate. This microbiota–gut–brain axis (MGBA) is crucial for normal development and physiology, and its dysregulation has been implicated in a range of neurological and intestinal disorders. Investigations into the mechanistic underpinnings of the MGBA have identified serotonin as a molecule of particular interest. In this review, we highlight recent advances toward understanding the role of endogenous serotonin in microbial communities, how microbial communities bidirectionally interact with host serotonin, and potential future engineering opportunities to leverage these novel mechanisms for biomedical applications.

Introduction
Serotonin is an evolutionarily ancient molecule found across all domains of life [1–7]. While typically regarded as a neurotransmitter, serotonin serves a diverse range of roles across disparate biological systems. In humans, serotonin production is actually highest in the gut where it is synthesized and secreted by enterochromaffin (EC) cells to modulate contractile frequency during peristalsis and regulate metabolism [1,2•]. In plants, serotonin is a signaling molecule that regulates growth and life-cycle progression and it also functions as a stress-defense molecule [3]. Beyond extracellular signaling, it is also known that proteins can be covalently modified with serotonin (serotonylation) with effects ranging from histone-dependent epigenetic modifications to regulation of platelet aggregation and the secretion of insulin [8]. Serotonin has also recently been shown to interact with membranes, altering their physical mechanics [9]. Taken together, it is becoming clearer that serotonin has a wider range of biological roles than is typically appreciated.

Multiple neurological and gastrointestinal diseases are associated with distinct microbiome signatures and impaired serotonergic function. Since bacteria in the gut can influence host serotonin levels, and vice versa, it is possible that microbiome activity may contribute to disease progression. In this review, we will highlight recent advances toward understanding the impacts of serotonin on bacterial physiology and microbiome composition, explore how these microbial changes could in turn influence host health, and describe recent engineering efforts that will be useful to fine-tune microbiome activity and could ultimately control serotonin signaling for therapeutic intervention (Figure 1). We will not discuss the existence of the microbiota–gut–brain axis (MGBA) beyond its influence on serotonin nor the regulatory roles serotonin plays in host physiology separate from the MGBA, as there are several excellent reviews on these topics [2•,10••–13•].

Evidence for serotonin as a signaling molecule in bacteria
Serotonin-biosynthesis pathways are well established in plants and animals but none have been described in bacteria, despite evidence that certain bacteria can synthesize serotonin [4–7]. However, exogenous serotonin was found to influence quorum sensing pathways and increase virulence in Pseudomonas aeruginosa. Specifically, micromolar concentrations of exogenous serotonin were shown to bind the quorum sensing transcription factor LasR and activate expression of multiple P. aeruginosa virulence genes. Further, it was shown that serotonin administration appeared to restore virulence in a P. aeruginosa lasI mutant, which is incapable of synthesizing the native quorum sensing molecule that activates LasR [14]. The opposite effect was observed in

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Serotonin signaling occurs bidirectionally between the host and members of the microbiome and may contribute to disease. Bacteria residing in the gut lumen can sense serotonin levels that can induce and/or inhibit gene expression related to biofilm formation, adhesion, motility, or virulence. The levels of luminal serotonin can also provide a selective advantage for certain species. Some microbiota can also increase luminal serotonin concentrations via increased expression of serotonin-synthesis enzymes, such as TpH1, or decreased expression of the SERT, and potentially other undefined mechanisms. These effects can be mediated by secreted bacterial secondary metabolites, or via interaction between the microbes and host receptors. Further, due to the existence of the gut–brain axis, microbiota can influence the levels of brain serotonin levels by modulating expression of serotonin, receptors, transporters, and synthesis, and metabolic enzymes, such as TpH2 and MAO. It is hypothesized that these microbiota-mediated changes in serotonin contribute to multiple neurological conditions.

In *Turicibacter sanguinis*, a member of the human microbiome, serotonin exposure was found to modulate the expression of 71 genes, resulting in downregulation of efflux and ABC transporters. Further, a novel serotonin transporter (SERT) was identified bioinformatically and validated using radiolabeled serotonin. This transporter could be blocked with the selective serotonin-reuptake inhibitor (SSRI), fluoxetine (Prozac). Fluoxetine co-administered with serotonin was shown to have an even broader impact on gene expression than serotonin, with differential expression observed for 395 genes. Interestingly, fluoxetine paired with serotonin was found to upregulate efflux and ABC transporters, suggesting fluoxetine can prevent and/or reverse serotonin-mediated changes in gene expression [16••]. These effects...
could be relevant to off-target effects of fluoxetine administration in humans where \( T. \) \textit{sanguinis} may be present in the microbiome. While there remain a limited number of in-depth investigations into the impact of serotonin on bacterial physiology, additional observational reports suggest that bacterial responses to serotonin may be widespread. For example, biofilm formation increased in the presence of serotonin in \textit{Enterococcus faecium} with modest improvements in adhesion [17]. The opposite was seen in \textit{Campylobacter jejuni} that was found to have reduced adhesion to colonic epithelial cells in the presence of serotonin [18]. It was also shown that exposure to serotonin in \textit{P. fluorescens} increased swimming and swarming motility and decreased siderophore production [19]. Taken together, while mechanistic data are lacking for many of these observations, they suggest some bacteria have a transcriptional response to serotonin.

These reports of exogenous serotonin influencing bacterial physiology coupled with evidence for serotonin biosynthesis in certain bacteria suggest that members of the microbiome could utilize serotonin as a signaling molecule. While many of the aforementioned species are not regarded as members of the microbiome, these findings warrant further investigations into the relationships between microbiota and serotonin. Further, while opportunistic pathogens such as \textit{P. aeruginosa} and EHEC are also not regarded as members of the human microbiome, they do colonize humans and cause disease, and therefore further study of their interactions with host serotonin may reveal routes of therapeutic intervention. It will be critical to systematically map these effects to understand how microbiome composition changes in response to altered levels of host serotonin and vice versa.

**Microbiota bidirectionally interact with host serotonin levels**

There is now evidence that the microbiome can alter host serotonin levels by inducing expression changes in serotonin synthesis, metabolism, secretion, or transport-related genes. In one example, it was found that humanized mice (germ-free mice colonized with human gut microbiota) were found to have elevated colonic levels of serotonin compared with germ-free mice. Furthermore, mRNA levels were increased for \textit{Tph1}, which encodes the rate-limiting enzyme of serotonin synthesis, and \textit{Chga}, which encodes a marker for serotonin secretion. Using an EC model cell line, it was shown that the increase in \textit{Tph1} mRNA was induced by the short-chain fatty acids acetate and butyrate [20]. Subsequent work showed that butyrate could rescue abnormal absorptive colonic motor activity in germ-free mice via mediating increased serotonin levels [21]. Similarly, acetate produced from human-derived \textit{Bifidobacterium dentium} was shown to elevate colonic levels of serotonin but to a lesser extent than treatment with \textit{B. dentium} or \textit{B. dentium} conditioned media. Interestingly, the group also showed monocolonized mice had increased expression of SERTs and receptors in the gut, as well as increased expression of the Htr2a receptor in the hippocampus, however, it remains to be shown whether this response is specific to \textit{B. dentium} [22•].

Similar broad impacts on serotoninergic gene expression were seen in the mouse gut and zebrafish brain using the probiotics \textit{Lactobacillus plantarum} [23] and \textit{Lactobacillus rhamnosus} [24], respectively. Increased SERT expression in the intestine has also been seen using \textit{Limosilactobacillus reuteri} and the supernatants of \textit{Bacillus subtilis}, \textit{Enterococcus faecium}, and \textit{Enterococcus faealis} [25,26]. It is important to note however, these effects are not conserved among bacteria, nor members of the human microbiome, as \textit{Bacteroides ovatus} did not recapitulate the increase of colonic serotonin seen with \textit{B. dentium} [22•].

Further, these effects are not limited to the secondary metabolites discussed. \textit{E. coli} Nissle 1917 was found to elevate host serotonin levels in a dose-dependent manner, which appeared to be independent from all secondary metabolites previously implicated in regulating serotoninergic gene expression [27].

In support of these findings, another study found that spore-forming bacteria were sufficient to promote serotonin synthesis in EC cells and that these effects were mediated by secondary metabolites. Specifically, the bile acids cholate and deoxycholate, as well as \textit{p}-aminobenzoate and tyramine, could induce elevated \textit{Tph1} mRNA and colonic serotonin. Butyrate was also seen to elevate \textit{Tph1} mRNA expression, but to a lesser extent. Importantly, direct administration of secondary metabolites only induced transient elevation of colonic serotonin levels, whereas colonization with spore-forming bacteria led to long-term elevation [28]. Furthermore, elevated intestinal serotonin resulted in enrichment of spore-forming bacteria, including \textit{T. sanguinis}. Interestingly, while \textit{T. sanguinis} colonization was impaired by fluoxetine exposure, fluoxetine had no impact on \textit{T. sanguinis} that had already been established, suggesting the import of serotonin may be necessary for successful colonization of the GI tract [16••].

It is also important to consider the levels of the serotonin precursor tryptophan and downstream metabolic products such as 5-hydroxyindoleacetic acid (HIAA) in MGBA crosstalk. It is well established that tryptophan can cross the blood–brain barrier [29] and, while serotonin has generally been regarded as unable to do so [30], evidence suggests serotonin may be effluxed from the brain to the blood [31]. Further, tryptophan metabolic pathways are highly enriched in prominent phyla of the human microbiome [32]. Bacterial extracellular...
Serotonin and the microbiota–gut–brain axis are linked to disease

Serotonin has been implicated in multiple disorders ranging from gastrointestinal diseases such as irritable bowel syndrome (IBS) to neurological diseases such as Parkinson's and Alzheimer's. In many such cases, there are also associated changes in microbiome composition, suggesting that MGBA-associated effects may be a component of disease progression. However, our understanding of the relationship between the MGBA, serotonin, and disease is still very much in its infancy and mechanistic data will be necessary to fully realize its scope. This section provides a summary of what is currently known about the relationships between disease and microbiome composition, and separately disease and serotonin, to focus continued research in this area.

IBS patients have altered levels of serotonin and the predominant symptoms, diarrhea or constipation, are correlated with reduced serotonin reuptake or release, respectively [38]. IBS patients can be differentiated from healthy controls with considerable accuracy by combining microbiome and metabolome data. Moreover, subtypes of IBS can be differentiated by levels of serotonin and its metabolite HIAA [39]. Similarly, serotonin levels in serum can be used to differentiate between active IBDs and refractory disease or remission [40]. One explanation for this may be Toll-like receptor-2 (TLR2) activity, which is activated during enteropathogenic infection and inhibits SERT activity, leading to elevated luminal serotonin concentrations, inducing inflammation and tissue damage [41]. In contrast, probiotics that increase colonic serotonin levels have already been successful in treating constipation [42]. Taken together, these findings highlight the importance of fine-tuning serotonin levels, so the appropriate response can be mounted.

In Parkinson's disease (PD), many associated symptoms, both motor and nonmotor, are related to degeneration of serotonin terminals [43]. A murine model of PD utilized the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, which increases α-synuclein expression in the ileum. The use of this neurotoxin was found to be associated with changes in gut microbiota and fecal transplants from treated mice to healthy mice resulted in decreases in serotonin and impaired motor function. Conversely, fecal transplants from healthy mice to treated mice restored serotonin levels and rescued motor function [44]. One hypothesis is that the decrease in serotonin is mediated by a reduction in short-chain fatty-acid (SCFA)-producing bacteria. PD patients have significant reductions of SCFA-producing bacteria in the gut microbiome [45] and SCFAs derived from gut microbes have been shown to stimulate serotonin production by EC cells [20].

Alzheimer's disease (AD) results in a profound impact on the serotonergic systems of the central nervous system, where overall serotonin content is decreased, and several serotonin receptors are substantially down-regulated [46]. Fecal microbiota transplant can modulate the accumulation of amyloid-β (Aβ), a hallmark of AD [47]. Fluoxetine and other SSRIs have been shown to decrease Aβ levels and/or plaques, and also modulate microbiome composition [16••,46]. Further, there is an ongoing phase-3 clinical trial with the drug GV-971 to improve cognition in AD patients. GV-971 was found to decrease Aβ-related pathologies, including reduction of neuroinflammation and improved cognition, and these effects were dependent on reconditioning of the gut microbiota [48•].

Multiple mouse models of autism-spectrum disorder (ASD), which similarly recapitulate social and behavioral features of the disorder, were shown to have striking differences in microbiome composition and impaired production of intestinal serotonin [48•,49]. In one model, treatment with Bacteroides fragilis corrected behavioral abnormalities and GI permeability [49]. In ASD, rare variants of the SERT gene are overexpressed, leading to hyperactivity of serotonin transport [50,51]. The most common variant was associated with rigid–compulsive behavior and sensory aversion [52,53], and when the mutation was cloned into mice, it induced many behaviors associated with ASD and also increased serotonin clearance and receptor sensitivity [54].

Anxiety and depression are common in many patients suffering from all of the aforementioned diseases.
associated metabolites, suppressing neuroinflammation, neurotrophically reduce colitis, depression, and anxiety-like behaviors were reduced in B. dentium monocolonized mice compared with germ-free mice, likely due to induction of serotonin production and expression of SERTs in the gut and receptors in the gut and brain [22•].

Toward microbiome engineering for control of serotonin levels
While there have not been any directed attempts to modulate host serotonin levels via microbiome engineering, much of what is now known about the influence of bacteria on host serotonergic systems have been uncovered by studying probiotic strains. As our understanding of the relationship between serotonin and microbiome-associated disease becomes clearer, it will be imperative to have tools to precisely modulate microbiome composition. For example, one could imagine the design of a microbial-based therapeutic that can sense host serotonin levels and respond accordingly to elevate or reduce these levels via secretion of secondary metabolites to up- or downregulate synthesis, metabolism, transport, or receptor gene expression.

Thus far, microbial therapeutics have been designed to sense a range of biomarkers and respond accordingly, and several engineered probiotics are currently in clinical trials for treating cancers, diabetes, inflammation, and metabolic disorders. For more in-depth information on these works, we direct you to these reviews [60,61]. For example, SYNB1618 is an engineered probiotic being used to reduce the levels of host metabolite phenylalanine in patients suffering from the metabolic disorder Phenylketonuria [62]. A similar concept could be used to degrade or synthesize serotonin without impacting the levels of other metabolites, such as SCFAs, which could have off-target consequences. As these engineered probiotics progress through clinical trials, advances are being made that will be crucial for widespread adoption of this novel therapeutic strategy. One such example is the specific targeting of an engineered probiotic to the small intestine, which could be subsequently activated by shining near-infrared light on the abdomen [63•]. This system was used to activate production of gamma-aminobutyric acid, granulocyte-colony-stimulating factor, or glucagon-like peptide-1 that alleviated anxiety-like behaviors, relieved PD symptoms, and excited neurons, respectively, in mice [63•]. This strategy could also be advantageous in the context of serotonin manipulation where, for example, increased serotonin production may be beneficial in the gut, but detrimental in off-target locations. Thus, while the use of engineered probiotics is still in its infancy, considerable advances will be made in this field as our basic scientific understanding of serotonin in the context of the MGBA continues to grow. Therefore, we are hopeful that these synergistic fields of research will move us toward manipulating serotonin signaling via the MGBA for therapeutic interventions in the coming years.

Conclusion
It is now clear that the human microbiome can affect host serotonergic systems and vice versa. The impact on gastrointestinal disorders can be readily appreciated and signaling via the MGBA further implicates this relationship in neurological diseases. It will be imperative to develop our understanding of this complex crosstalk by further investigating the impact of serotonin exposure on bacteria, how serotonin can impact microbial competition and microbiome composition, the mechanisms by which bacterial secondary metabolites can act on serotonergic machinery, and the extent to which the MGBA is implicated in disease. By defining the precise nature of these relationships, microbial engineering strategies can be utilized to design therapeutic interventions and ultimately treat human disease.

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest
•• of outstanding interest.


This review provides an overview of recent progress toward understanding the role of peripheral serotonin, touching on traditional roles in the central and enteric nervous systems, involvement in metabolic homeostasis and the MGBA.


This review provides comprehensive and in-depth summary of the advances made toward understanding the MGBA.


This review provides an overview of the recent work implicating serotonin in metabolism.


This review provides an overview of the recent work implicating serotonin in inflammation.


An important paper demonstrating serotonin can act as a signaling molecule but via a distinct, two-component sensing mechanism.


Important work demonstrating the bidirectional influence between the microbe and host serotonin levels. The authors also report the first identified bacterial serotonin transporter.


A paper providing mechanistic evidence as to how bacteria can modulate serotoninergic gene expression.


A study demonstrating bacterial EVs can modulate serotonergic gene expression, possibly by crossing the blood-brain barrier.


Important work demonstrating evidence for the therapeutic action of GV-971 in treating AD, which is now in phase 3 clinical trials.


Interesting article where the authors use optogenetics to control the therapeutic action of engeiered probiotics.