Molecular Cell

CellPress

Perspective

Toward Understanding Microbiome-Neuronal Signaling

K.G. Jameson,[1,*] C.A. Olson,[1] S.A. Kazmi,[1] and E.Y. Hsiao[1]
[1]Department of Integrative Biology and Physiology, University of California, Los Angeles, Los Angeles, CA 90095, USA
*Correspondence: kgjameson@g.ucla.edu
https://doi.org/10.1016/j.molcel.2020.03.006

Host-associated microbiomes are emerging as important modifiers of brain activity and behavior. Metabolic, immune, and neuronal pathways are proposed to mediate communication across the so-called microbiota-gut-brain axis. However, strong mechanistic evidence, especially for direct signaling between microbes and sensory neurons, is lacking. Here, we discuss microbial regulation of short-chain fatty acids, neurotransmitters, as-yet-uncharacterized biochemicals, and derivatives of neuromodulatory drugs as important areas for assessing microbial interactions with the nervous system.

We have co-evolved with trillions of indigenous microorganisms that comprise the human microbiota. Over the past decade, the notion that the microbiome is a key regulator of host physiology and behavior has skyrocketed with the advancement of multi-omics technologies, gnotobiotic tools, intersectional genetics, and live imaging. Early studies linking alterations in the gut microbiome with neurobehavioral phenotypes launched the concept of a microbiota-gut-brain axis whereby intestinal microbes influence brain and behavior through immune, neuronal, and metabolic pathways. In particular, emerging evidence suggests that select members of the microbiota have the ability to synthesize and/or regulate various neurochemicals known to modulate neurotransmission as well as a vast milieu of other metabolites that may directly or indirectly impact neuronal activity. As such, the role of mutualistic microbes in regulating sensory neuronal communication along the gut-brain axis is of active scientific interest. Microbial modulation of dietary molecules, neurotransmitters, as-yet-uncharacterized metabolites, and neurological drugs represent major areas for research toward uncovering mechanisms for microbial modulation of neuronal activity (Figure 1).

Microbial Regulation of Short-Chain Fatty Acids

Early studies on feeding behavior, led by such pioneers as Claude Bernard and Ivan Pavlov, laid the foundation for the concept of a gut-brain axis through dietary modulation (Leulier et al., 2017). With the advent of germ-free rodent models in the 1920s (Gustafsson, 1946-1947), gut microbes were identified as important mediators of dietary metabolism and host nutrition. Germ-free animals exhibited substantially deficient levels of the short-chain fatty acids (SCFAs) butyrate, propionate, acetate, and valerate in the intestine and blood, indicating a crucial role for the microbiota in regulating local and systemic SCFA bioavailability in the host (Høverstad and Midtvedt, 1986). Continued research in this area has uncovered molecular mechanisms underlying microbial production of SCFAs through their fermentation of complex polysaccharides, propelled by the discovery and characterization of polysaccharide utilization loci present in *Bacteroidetes* (Bjursell et al., 2006).

A wealth of evidence has further demonstrated that alterations in the microbiota and SCFAs are associated with conditions in which food intake behaviors are dysregulated (Byrne et al., 2015). In particular, alterations in the gut microbiota are seen in obese mice and humans, which correlate with alterations in the levels of acetate and butyrate (Ridaura et al., 2013; Turnbaugh et al., 2006). Propionate administration to patients with obesity enhanced gut hormone secretion while reducing adiposity and overall weight gain (Chambers et al., 2015). While some of the animal studies highlight microbial regulation of appetite as a basis for the observed differences in weight gain, exactly how microbial regulation of SCFAs impacts host feeding behaviors remains unclear. The SCFA free fatty acid receptors 2 and 3 (FFAR2 and FFAR3, respectively) are expressed in the enteric nervous system and the portal nerve, as well as various sensory ganglia (De Vadder et al., 2014; Egerod et al., 2018), suggesting a role for activation of the nervous system in mediating these effects. Consistent with this, propionate feeding induces *fos* expression in the dorsal vagal complex of the brainstem, the hypothalamus, and the spinal cord (De Vadder et al., 2014), raising the question of whether SCFA-induced stimulation of peripheral sensory neuronal activity could mediate the effects of SCFAs on host feeding behavior.

As the list of host behaviors that are modified by the gut microbiome continues to grow (Vuong et al., 2017), a key open question is the extent to which the microbial regulation of molecules relating broadly to nutrition underlies the reported effects of the microbiome on complex host behaviors, spanning homeostatic feeding and social, stress-related, and cognitive domains. SCFAs are fundamental molecules involved in regulating energy homeostasis, and SCFA receptors are expressed by a wide variety of non-neuronal cell subtypes as well. In immune cells, for example, SCFAs can regulate T regulatory cell differentiation (Arpaia et al., 2013; Furusawa et al., 2013; Smith et al., 2013) and microglial maturation (Erny et al., 2017), whereas in enteroendocrine cells, SCFAs can stimulate the release of gut hormones (Larraufie et al., 2018). In addition to promoting SCFAs, the gut microbiota is integral to secondary metabolism of bile acids, another class of diet-related metabolites for which cognate receptors are expressed by various cell types, including

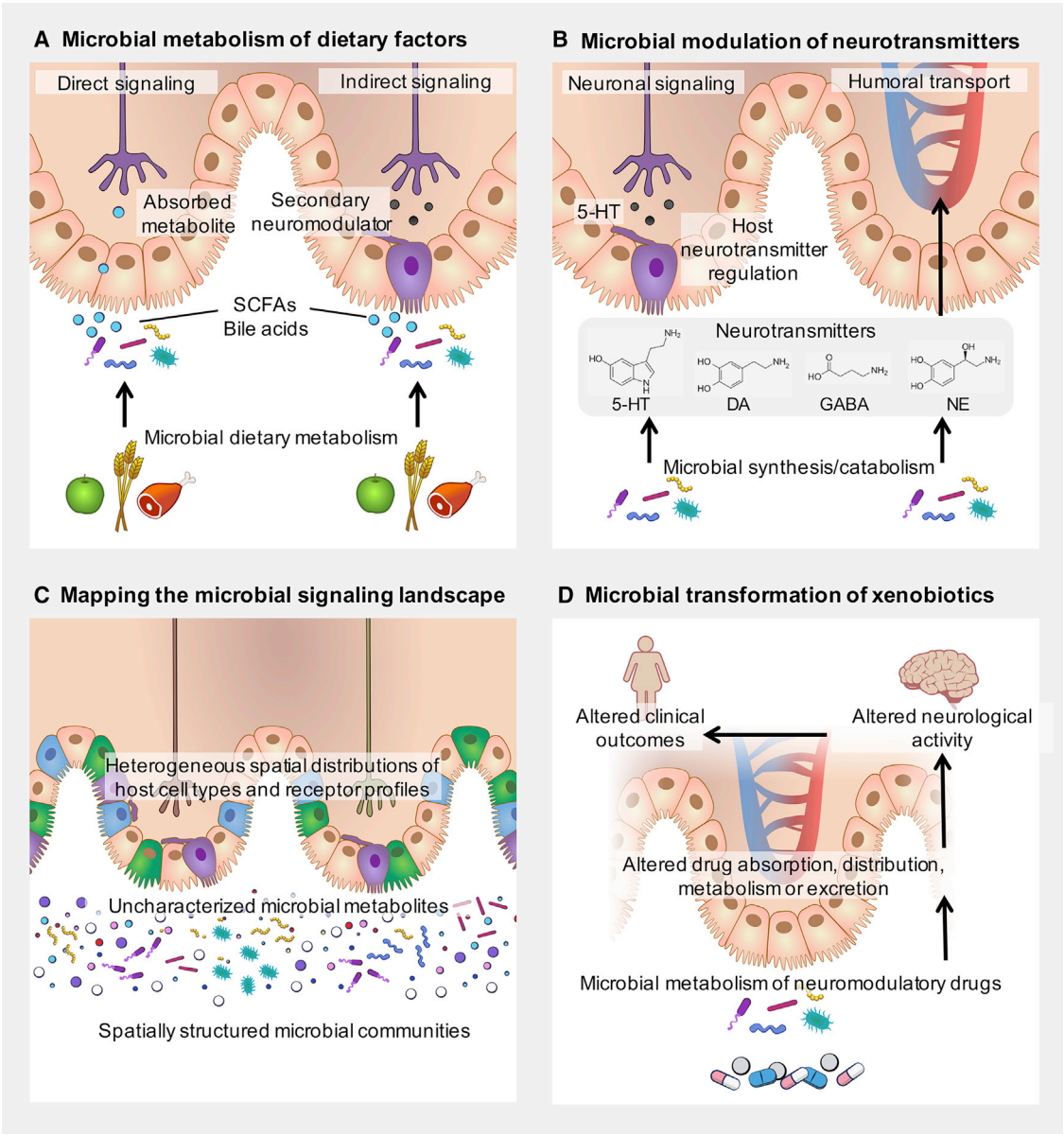

Figure 1. Microbial Interactions with the Nervous System through the Regulation of Dietary Products, Neurotransmitters, Uncharacterized Biochemicals, and Neuromodulatory Drugs

(A) Select bacteria from the gut microbiota produce short-chain fatty acids (SCFAs) and modify bile acids through dietary metabolism. Metabolites from the microbiota can signal directly to mucosal afferent fibers of sensory neurons (left) or can signal to neurons via intermediate interactions with enteroendocrine or epithelial cells (right).

(B) Select bacteria from the gut microbiota can directly synthesize, consume, or sense neurotransmitters such as serotonin (5-HT), dopamine (DA), gamma-aminobutyric acid (GABA), and norepinephrine (NE) (center) or regulate host biosynthesis of neurotransmitters, like serotonin (5-HT) (center left). Microbially modulated neurotransmitters have the potential to interact with sensory neurons (left) or be circulated humorally (right) to reach the blood-brain barrier.

(C) The physiological landscape for microbial interactions with the nervous system is complex. Emerging evidence suggests that microbial communities are spatially structured (bottom), which yields "microbiogeographies" that vary in physiological function. In addition, the microbiome regulates various metabolites in the host, many of which remain uncharacterized (center). Further complexity is introduced when considering the heterogeneity of host cell types within the intestine, spanning various types of epithelial, endocrine, immune, and neuronal cells that are also spatially distributed and can vary temporally in their localization via turnover and remodeling. Spatial maps of signaling receptors, especially those available for mediating neural communication across intestinal cell types, will help inform functional pathways for microbe-host interactions.

(D) The microbiome is increasingly appreciated as an important modulator of xenobiotic metabolism, particularly for neuromodulatory drugs, including anti-psychotics, anticholinergics, antidepressants, and opioids. Microbial transformation of drugs for neurological conditions could alter their absorption, distribution, metabolism, and/or excretion in the host, with potential downstream consequences on host neural activity and symptoms of neurological disease.

subsets of sensory neurons, to regulate diverse host phenotypes (Mertens et al., 2017). In light of their pleiotropic effects, studies that dissect the precise signaling pathways by which SCFAs and bile acids alter host behaviors are warranted. Efforts to determine the functional roles of specific neuronal pathways in SCFA and bile acid signaling would be particularly illuminating toward uncovering roles for the microbiota in regulating neuronal activity via dietary metabolism.

Microbial Regulation of Neurochemicals

While the gut microbiota may affect host behavior through the regulation of dietary metabolites, like SCFAs and bile acids, emerging research indicates that select gut microbes also regulate levels of host neurotransmitters. The finding that microbes can synthesize neurotransmitters is rooted in the first discovery of chemical transmitters by Sir Henry Dale in the early 1900s (Valenstein, 2002). In studying ergot on wheat rye, he discovered the transmitter acetylcholine over a decade before it was extracted from mammalian tissue. Together with George Barger, Dale found that acetylcholine mimicked the effects of parasympathetic nerve stimulation, suggesting chemically mediated neurotransmission. It was realized later that the acetylcholine itself was likely derived from *Bacillus* contaminants in the ergot rather than from the ergot itself. Since this landmark discovery, additional neurotransmitters, including norepinephrine (NE), serotonin (5-HT), γ-aminobutyric acid (GABA), and dopamine (DA) have been found to be produced by bacteria in culture and to be regulated by the microbiota in animals (Strandwitz, 2018). Despite these tantalizing associations, all kingdoms of life produce the amino-acid derivatives that form common "neurotransmitters," raising the questions: What are the functional roles of neurotransmitters in microbes, and can host-associated microbes impact the nervous system through neurotransmitter modulation?

As yet, only a few studies have examined the effects of canonical neurotransmitters on bacterial physiology. One relatively early series of studies revealed that the catecholamines NE and epinephrine exhibit a structural similarity to the quorum-sensing molecule autoinducer-3 and that, therefore, each stimulates enterohemorrhagic *E. coli* motility and virulence (Clarke et al., 2006). Researchers hypothesize that this direct effect of NE and epinephrine on bacterial pathogenesis may contribute to the ability of stress to increase susceptibility to infection. More recently, a study utilizing *in vitro* co-culture screens and metagenomic datasets revealed GABA-producing versus GABA-consuming bacteria from the human gut microbiota (Strandwitz et al., 2019). In particular, GABA synthesized by *Bacteroides fragilis* supported the growth of KLE1738, suggesting that select neurotransmitters may serve as growth substrates for bacteria. A separate study found that 5-HT promotes intestinal colonization of the bacterium *Turicibacter sanguinis*, similarly suggesting a role for a neurotransmitter in promoting microbial fitness (Fung et al., 2019). Beyond these initial findings, little is known regarding the extent of neurotransmitter modulation across various members of the gut microbiota, the specific microbial genes and gene products used for their synthesis and catabolism, and the molecular pathways underlying microbial sensing and response to neurotransmitters. Integrated microbiological, biochemical, and bioinformatic approaches are needed to support *in silico* predictions informed by multi-omic datasets, *in vitro* determination of microbial gene and protein function, and *in vivo* investigation of microbial community responses. Identifying the molecular underpinnings for microbial synthesis, transformation, and physiological response to neurotransmitters would further enable mechanistic interrogation of the potential consequences of microbiota-dependent neurotransmitter modulation on host physiology.

Despite evidence that select host-associated bacteria regulate neurotransmitter levels locally in the intestine and, in some cases, systemically in the blood or distantly in the brain itself, whether microbial modulation of neurotransmitters actually influences neuronal activity and behavior remains poorly understood. In mice, the gut microbiota is responsible for promoting the biosynthesis of up to 60% of colonic and blood 5-HT levels by enterochromaffin cells (ECs) in the intestinal epithelium (Yano et al., 2015). In the intestine, microbially modulated 5-HT activates intrinsic afferent primary neurons of the myenteric plexus to promote gastrointestinal motility, but whether extrinsic intestinally innervating nerves are also affected remains unknown. Separate studies suggest that subsets of ECs may synapse with 5-HT-receptive afferent fibers of chemosensory vagal or dorsal root neurons (Bellono et al., 2017; Bohórquez et al., 2015), suggesting a direct path for microbial regulation of local 5-HT to impact the central nervous system. While evidence for microbiome-gut-sensory neuronal signaling is currently lacking, a growing number of studies reporting effects of the microbiome on host behavior have applied subdiaphragmatic vagotomy to demonstrate that severely impaired vagal signaling abrogates microbial effects on behavior (Bravo et al., 2011; Sgritta et al., 2019). Additional studies that circumvent the confounds of vagotomy and that carefully examine functional neuronal responses to microbially modulated neurochemicals are needed to evaluate the potential for microbes to directly affect neural activity. These efforts would be aided greatly by the development of synthetic biological tools to identify, regulate, and manipulate microbial genes for neurochemical modulation, coupled with host gnotobiotic and intersectional genetic tools for selective microbial colonization and targeted neurophysiological assessments. In addition to evaluating sensory neuronal pathways, efforts to examine the humoral transport of microbially modulated neurochemicals or their precursors are warranted. Consistent with this possibility, heavy-isotope-labeled acetate in the colon enters the bloodstream, crosses the blood-brain barrier, elevates hypothalamic acetate, and feeds into GABA neuroglial cycling to increase central GABA production (Frost et al., 2014). Novel tools to selectively label target neuromodulators that are produced or regulated by the microbiota, along with technologies for spatiotemporal tracking in animals, would help enable efforts to evaluate the ability of the microbiota to impact distant sites in the central nervous system.

Uncharacterized Microbial Products and the Nervous System

Aside from SCFAs, bile acids, and neurotransmitters, there are likely many additional microbiota-dependent biochemicals that have the potential to interact with neurons. The human

microbiota regulates a vast repertoire of metabolites not only in the intestinal lumen but also in the circulating blood and various organ systems of the host. However, the identity, cognate receptors, signaling pathways, and physiological functions of many microbially modulated metabolites remain poorly understood (Milshteyn et al., 2018). Recent functional metagenomics studies have begun to reveal the scope of bacterial genes for metabolite synthesis and signaling to the host. By screening cosmid metagenomic libraries, researchers identified host-associated bacterial effector genes, which, upon bioassaying the gene products, resulted in the discovery of commendamide, an N-acyl amide capable of activating the host G-protein-coupled receptor (GPCR; also referred to as GPR) G2A (Cohen et al., 2015). Continuing their work on N-acyl amides, the researchers also demonstrated that bacterially produced N-acyl serinol activated the endocannabinoid receptor GPR119A (Cohen et al., 2017). These studies illustrate that functional metagenomics can be a powerful tool to not only discover novel bacterial metabolites but also reveal how bacterial metabolites can affect host physiology through mimicking endogenous GPCR ligands.

Recent studies have begun to identify GPCRs and orphan receptors that are activated by bacterial metabolites *in vitro*. In a screen of supernatants from individually cultured bacteria from the human gut microbiota, receptors for DA, histamine, and 5-HT were highly responsive to soluble bacterial products. Among many additional candidates, bacterially derived phenethylamine and tyramine activated DA receptors, while bacterial production of histamine itself activated histamine receptors (Chen et al., 2019). In addition to these, as-yet-unidentified bacterial products activated a wide range of other neuropeptide and hormone receptors, classically known to be expressed in the nervous system. In another study, fractionated supernatants from a simplified human microbiome consortium were similarly found to robustly activate neurotransmitter GPCRs. In addition to histamine itself, bacterially produced cadaverine, putrescine, and agmatine also activated histamine receptors (Colosimo et al., 2019). Bacterial supernatants containing 9,10-methylenehexadecanoic acid activated brain angiogenesis factor 1, while 12-methyltetradecanoic acid activated neuromedin receptor 1. Overall, these studies provide proof of concept that select microbial products could activate GPCRs known to be expressed by neurons.

Further research is required to identify specific microbial metabolites that are capable of signaling to neurons and to determine whether they are bioavailable to the host when produced by microbes within complex host-associated communities. While existing studies demonstrate the potential for bacteria to activate select GPCRs, the authentic identities of the bacterial molecules that affect individual receptors remain largely unknown. Additionally, our knowledge as yet relies primarily on bacteria grown in culture, alone or in limited communities, raising the question of whether there are additional molecules left unassayed from microbes that were not cultured and whether the data capture physiologically relevant outputs of complex microbial community interactions. Culture-independent approaches to screen metabolites directly from host biospecimens would greatly aid in this regard. Beyond bacteria from the microbiome, the roles for the mycobiome and virome in altering neuronal activity remain understudied. While sensory nerve fibers in the skin directly sense infectious *Candida albicans* (Kashem et al., 2015), whether non-pathogenic members of the mycobiome influence neuronal activity is poorly understood. Moreover, bacteriophages alter levels of the neurotransmitters tryptamine and tyramine in the gut (Hsu et al., 2019), but whether these alterations ultimately impact neuronal activity is unclear. These studies highlight a need for novel tools to selectively modulate non-bacterial members of the microbiome in order to fully understand the complex role of the entire microbiome in modulating the host nervous system.

While initial evidence suggests that microbes are capable of synthesizing molecules that could directly bind to neuron-relevant GPCRs *in vitro*, additional research is needed to determine whether they bind neuronal GPCRs in host tissues and to further evaluate the physiological consequences of their signaling. Accordingly, greater attention to spatial variations in metabolite production and receptor activation *in vivo* is warranted. Microbial communities exhibit distinct spatial structures, or "microbiogeographies," along and across the gastrointestinal tract (Donaldson et al., 2016). In addition, recent single-cell RNA-sequencing studies suggest that there is cellular, and potentially spatial, heterogeneity in the receptor profiles of intestinally innervating dorsal root and vagal neurons (Hockley et al., 2019; Kupari et al., 2019). Advances in technologies for high-throughput *in situ* microbial imaging, metabolite profiling, and GPCR mapping would help to establish the physiological landscape of the intestine to inform functional microbiome-nervous system interactions.

Microbial Interactions with Drugs for Neurological Disease

The finding that select microbes can synthesize, modulate, sense, and/or respond to neurochemicals raises the question of whether they would additionally interact with medical drugs that modulate neurotransmission. The gut microbiota encodes a diverse array of enzymes capable of metabolizing pharmacological agents, thus potentially influencing their bioavailability to the host and contributing to the wide range of intra-patient variability in drug efficacy. Early work describing how the process of glucuronidation promotes drug clearance, coupled with the identification of bacterial beta-glucuronidases from gut microbes, have set the foundation for pioneering studies on microbiomes as modulators of xenobiotic metabolism (Wallace et al., 2010). Since then, xenobiotic metabolism by the microbiome has been extended to numerous drugs targeting neurological indications. In sequencing studies of the human microbiota and culture-based screens of bacterial interactions with common medications, many antipsychotics, antidepressants, opioids, and anticholinergic drugs greatly affected bacterial physiology and correlated with alterations in the composition of the gut microbiota (Jackson et al., 2018; Maier et al., 2018; Zimmermann et al., 2019a). While the distinct contribution of the microbiota to the metabolism of drugs can be difficult to quantify alongside host-derived enzymes carrying out the same metabolic functions, a recent study utilized gnotobiotic, pharmacological, and bacterial genetic approaches to disentangle microbial versus host xenobiotic transformations.

By comparing the metabolism of the antiviral drug brivudine in multiple tissues of germ-free mice that vary in a single microbiome-encoded enzyme, researchers were able to generate a pharmacokinetic model to predict the contribution of the microbiota to features of drug metabolism, including oral bioavailability, host drug-metabolizing activity, metabolite absorption, and intestinal transit (Zimmermann et al., 2019b). This modeling approach was further applied to dissect microbiota contributions to the metabolism of the antiviral drug sorivudine and the benzodiazepine clonazepam (Zimmermann et al., 2019b).

Separate studies have utilized biochemical and metagenomic approaches to identify particular bacterial species and novel bacterial enzymes that modulate the metabolism of drugs, including those for neurodegenerative diseases. The mainstay treatment for Parkinson's disease, levodopa (L-dopa), is a natural precursor of DA that, when administered peripherally, is able to cross the blood-brain barrier for local conversion to DA in the brain. However, the gastrointestinal tract is a site of extensive metabolism of the drug, leading to reduced bioavailability and unwanted side effects caused by elevations in peripheral DA. Informed by mechanisms for host metabolism of DA, a recent study identified a novel interspecies pathway for microbial metabolism of L-dopa, whereby *Enterococcus faecalis* decarboxylates L-dopa to DA, which is subsequently dehydroxylated by *Eggerthella lenta* to m-tyramine (Maini Rekdal et al., 2019). Remarkably, the presence of a single-nucleotide polymorphism in the bacterial gene encoding dopamine dehydroxylase was predictive of the capacity for certain patients to metabolize the drug. As such, the field has begun to appreciate the microbiota as a potential therapeutic target not only to aid in drug efficacy for the treatment of neurological disorders but also as a means for developing additional personalized medical treatments.

Despite these exciting advancements toward our understanding of the molecular mechanisms behind the microbial metabolism of neuromodulatory drugs, a gap remains in our understanding of the relevance of these findings to the clinic. Few, if any, studies to date have rigorously assessed the symptomatic outcomes resulting from altering the microbial metabolism of drugs for neurological disorders. As a result, it remains unclear whether these mechanisms are ultimately impactful for clinically relevant outcomes in the host. Experiments utilizing genetically tractable bacterial species alongside gnotobiotic tools in animal models of disease are needed to assess the role of microbe-specific functions on drug bioavailability and neurobehavioral outcomes. Advancements such as these are paramount for our ability to better understand roles for the microbiome in regulating inter-patient variability in responsiveness to drugs for neurological conditions and to assess the potential to inform tractable strategies for clinical intervention.

Conclusions

A growing body of evidence indicates that disruptions in host-associated microbiomes can modify animal behavior and further supports the notion of signaling across a microbiota-gut-brain axis. To date, several studies highlight sensory neuronal signaling, humoral metabolic communication, and immunomodulation as likely direct and indirect pathways that mediate microbiota-nervous system interactions, but studies that clearly evaluate and dissect these signaling mechanisms are lacking. Recent advances in sequencing, viral targeting, and intersectional genetic and imaging tools, combined with gnotobiotic and bacterial genetic systems, can better our understanding of the molecular and cellular mechanisms underlying microbiota-gut-brain communication and the nuances that arise from the coordinated signaling of heterogeneous cell types in response to pleiotropic microbial cues. In particular, studies profiling sensory neurons and intestinal epithelial cells have uncovered the possibility for both direct and indirect activation of sensory neurons by microbiota-dependent dietary products, neurotransmitters, and as-yet-uncharacterized metabolites either through binding of receptors on afferent fibers themselves or via signaling to enteroendocrine cells in the gut epithelium. However, experiments that use conditional receptor knockouts in specific neuronal or epithelial subpopulations and gain- or loss-of-function constructs in bacteria may aid in identifying pathway-specific effects of microbial signals in regulating host brain function and behavior. Additionally, few studies to date have used *in vivo* electrophysiological- and geneticallyencoded calcium-indicator-based tools to directly assess the functional role of microbial-metabolite effects on neuronal activity. Understanding the distinct circuitry and functional signatures involved in mediating neuronal communication along the gut-brain axis is imperative for our understanding of how the gut microbiota modifies host physiology. While such studies can be performed in animal models, an added challenge is in assessing the relevance of findings to human health outcomes. Interrogating whether microbes from the human microbiota interact with neuromodulatory drugs, and whether such interactions have measurable consequences on drug efficacy and clinical outcomes, may serve a tractable context. Overall, the future offers the exciting prospect of uncovering fundamental principles for how microbes and microbial products are detected and interpreted by host sensory systems, toward understanding the co-evolution of animals with their associated microbiomes.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., Liu, H., Cross, J.R., Pfeffer, K., Coffer, P.J., and Rudensky, A.Y. (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature *504*, 451–455.

Bellono, N.W., Bayrer, J.R., Leitch, D.B., Castro, J., Zhang, C., O'Donnell, T.A., Brierley, S.M., Ingraham, H.A., and Julius, D. (2017). Enterochromaffin cells are gut chemosensors that couple to sensory neural pathways. Cell *170*, 185–198.e16.

Bjursell, M.K., Martens, E.C., and Gordon, J.I. (2006). Functional genomic and metabolic studies of the adaptations of a prominent adult human gut symbiont, Bacteroides thetaiotaomicron, to the suckling period. J. Biol. Chem. *281*, 36269–36279.

Bohórquez, D.V., Shahid, R.A., Erdmann, A., Kreger, A.M., Wang, Y., Calakos, N., Wang, F., and Liddle, R.A. (2015). Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. J. Clin. Invest. *125*, 782–786.

Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., Bienenstock, J., and Cryan, J.F. (2011). Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc. Natl. Acad. Sci. USA *108*, 16050–16055.

Byrne, C.S., Chambers, E.S., Morrison, D.J., and Frost, G. (2015). The role of short chain fatty acids in appetite regulation and energy homeostasis. Int. J. Obes. *39*, 1331–1338.

Chambers, E.S., Viardot, A., Psichas, A., Morrison, D.J., Murphy, K.G., Zac-Varghese, S.E., MacDougall, K., Preston, T., Tedford, C., Finlayson, G.S., et al. (2015). Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. Gut *64*, 1744–1754.

Chen, H., Nwe, P.K., Yang, Y., Rosen, C.E., Bielecka, A.A., Kuchroo, M., Cline, G.W., Kruse, A.C., Ring, A.M., Crawford, J.M., et al. (2019). A forward chemical genetic screen reveals gut microbiota metabolites that modulate host physiology. Cell *177*, 1217–1231.e18.

Clarke, M.B., Hughes, D.T., Zhu, C., Boedeker, E.C., and Sperandio, V. (2006). The QseC sensor kinase: a bacterial adrenergic receptor. Proc. Natl. Acad. Sci. USA *103*, 10420–10425.

Cohen, L.J., Kang, H.S., Chu, J., Huang, Y.H., Gordon, E.A., Reddy, B.V., Ternei, M.A., Craig, J.W., and Brady, S.F. (2015). Functional metagenomic discovery of bacterial effectors in the human microbiome and isolation of commendamide, a GPCR G2A/132 agonist. Proc. Natl. Acad. Sci. USA *112*, E4825–E4834.

Cohen, L.J., Esterhazy, D., Kim, S.H., Lemetre, C., Aguilar, R.R., Gordon, E.A., Pickard, A.J., Cross, J.R., Emiliano, A.B., Han, S.M., et al. (2017). Commensal bacteria make GPCR ligands that mimic human signalling molecules. Nature *549*, 48–53.

Colosimo, D.A., Kohn, J.A., Luo, P.M., Piscotta, F.J., Han, S.M., Pickard, A.J., Rao, A., Cross, J.R., Cohen, L.J., and Brady, S.F. (2019). Mapping interactions of microbial metabolites with human G-protein-coupled receptors. Cell Host Microbe *26*, 273–282.e7.

De Vadder, F., Kovatcheva-Datchary, P., Goncalves, D., Vinera, J., Zitoun, C., Duchampt, A., Bäckhed, F., and Mithieux, G. (2014). Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. Cell *156*, 84–96.

Donaldson, G.P., Lee, S.M., and Mazmanian, S.K. (2016). Gut biogeography of the bacterial microbiota. Nat. Rev. Microbiol. *14*, 20–32.

Egerod, K.L., Petersen, N., Timshel, P.N., Rekling, J.C., Wang, Y., Liu, Q., Schwartz, T.W., and Gautron, L. (2018). Profiling of G protein-coupled receptors in vagal afferents reveals novel gut-to-brain sensing mechanisms. Mol. Metab. *12*, 62–75.

Erny, D., Hrabě de Angelis, A.L., and Prinz, M. (2017). Communicating systems in the body: how microbiota and microglia cooperate. Immunology *150*, 7–15.

Frost, G., Sleeth, M.L., Sahuri-Arisoylu, M., Lizarbe, B., Cerdan, S., Brody, L., Anastasovska, J., Ghourab, S., Hankir, M., Zhang, S., et al. (2014). The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. Nat. Commun. *5*, 3611.

Fung, T.C., Vuong, H.E., Luna, C.D.G., Pronovost, G.N., Aleksandrova, A.A., Riley, N.G., Vavilina, A., McGinn, J., Rendon, T., Forrest, L.R., and Hsiao, E.Y. (2019). Intestinal serotonin and fluoxetine exposure modulate bacterial colonization in the gut. Nat. Microbiol. *4*, 2064–2073.

Furusawa, Y., Obata, Y., Fukuda, S., Endo, T.A., Nakato, G., Takahashi, D., Nakanishi, Y., Uetake, C., Kato, K., Kato, T., et al. (2013). Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature *504*, 446–450.

Gustafsson, B. (1946-1947). Germ-free rearing of rats. Acta Anat. (Basel) *2*, 376–391.

Hockley, J.R.F., Taylor, T.S., Callejo, G., Wilbrey, A.L., Gutteridge, A., Bach, K., Winchester, W.J., Bulmer, D.C., McMurray, G., and Smith, E.S.J. (2019). Single-cell RNAseq reveals seven classes of colonic sensory neuron. Gut *68*, 633–644.

Høverstad, T., and Midtvedt, T. (1986). Short-chain fatty acids in germfree mice and rats. J. Nutr. *116*, 1772–1776.

Hsu, B.B., Gibson, T.E., Yeliseyev, V., Liu, Q., Lyon, L., Bry, L., Silver, P.A., and Gerber, G.K. (2019). Dynamic modulation of the gut microbiota and metabolome by bacteriophages in a mouse model. Cell Host Microbe *25*, 803–814.e5.

Jackson, M.A., Verdi, S., Maxan, M.E., Shin, C.M., Zierer, J., Bowyer, R.C.E., Martin, T., Williams, F.M.K., Menni, C., Bell, J.T., et al. (2018). Gut microbiota associations with common diseases and prescription medications in a population-based cohort. Nat. Commun. *9*, 2655.

Kashem, S.W., Riedl, M.S., Yao, C., Honda, C.N., Vulchanova, L., and Kaplan, D.H. (2015). Nociceptive sensory fibers drive interleukin-23 production from CD301b+ dermal dendritic cells and drive protective cutaneous immunity. Immunity *43*, 515–526.

Kupari, J., Häring, M., Agirre, E., Castelo-Branco, G., and Ernfors, P. (2019). An atlas of vagal sensory neurons and their molecular specialization. Cell Rep. *27*, 2508–2523.e4.

Larraufie, P., Martin-Gallausiaux, C., Lapaque, N., Dore, J., Gribble, F.M., Reimann, F., and Blottiere, H.M. (2018). SCFAs strongly stimulate PYY production in human enteroendocrine cells. Sci. Rep. *8*, 74.

Leulier, F., MacNeil, L.T., Lee, W.J., Rawls, J.F., Cani, P.D., Schwarzer, M., Zhao, L., and Simpson, S.J. (2017). Integrative physiology: at the crossroads of nutrition, microbiota, animal physiology, and human health. Cell Metab. *25*, 522–534.

Maier, L., Pruteanu, M., Kuhn, M., Zeller, G., Telzerow, A., Anderson, E.E., Brochado, A.R., Fernandez, K.C., Dose, H., Mori, H., et al. (2018). Extensive impact of non-antibiotic drugs on human gut bacteria. Nature *555*, 623–628.

Maini Rekdal, V., Bess, E.N., Bisanz, J.E., Turnbaugh, P.J., and Balskus, E.P. (2019). Discovery and inhibition of an interspecies gut bacterial pathway for Levodopa metabolism. Science *364*, eaau6323.

Mertens, K.L., Kalsbeek, A., Soeters, M.R., and Eggink, H.M. (2017). Bile acid signaling pathways from the enterohepatic circulation to the central nervous system. Front. Neurosci. *11*, 617.

Milshteyn, A., Colosimo, D.A., and Brady, S.F. (2018). Accessing bioactive natural products from the human microbiome. Cell Host Microbe *23*, 725–736.

Ridaura, V.K., Faith, J.J., Rey, F.E., Cheng, J., Duncan, A.E., Kau, A.L., Griffin, N.W., Lombard, V., Henrissat, B., Bain, J.R., et al. (2013). Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science *341*, 1241214.

Sgritta, M., Dooling, S.W., Buffington, S.A., Momin, E.N., Francis, M.B., Britton, R.A., and Costa-Mattioli, M. (2019). Mechanisms underlying microbial-mediated changes in social behavior in mouse models of autism spectrum disorder. Neuron *101*, 246–259.e6.

Smith, P.M., Howitt, M.R., Panikov, N., Michaud, M., Gallini, C.A., Bohlooly-Y, M., Glickman, J.N., and Garrett, W.S. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science *341*, 569–573.

Strandwitz, P. (2018). Neurotransmitter modulation by the gut microbiota. Brain Res. *1693* (Pt B), 128–133.

Strandwitz, P., Kim, K.H., Terekhova, D., Liu, J.K., Sharma, A., Levering, J., McDonald, D., Dietrich, D., Ramadhar, T.R., Lekbua, A., et al. (2019). GABA-modulating bacteria of the human gut microbiota. Nat. Microbiol. *4*, 396–403.

Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., and Gordon, J.I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. Nature *444*, 1027–1031.

Valenstein, E.S. (2002). The discovery of chemical neurotransmitters. Brain Cogn. *49*, 73–95.

Vuong, H.E., Yano, J.M., Fung, T.C., and Hsiao, E.Y. (2017). The microbiome and host behavior. Annu. Rev. Neurosci. *40*, 21–49.

Wallace, B.D., Wang, H., Lane, K.T., Scott, J.E., Orans, J., Koo, J.S., Venkatesh, M., Jobin, C., Yeh, L.A., Mani, S., and Redinbo, M.R. (2010). Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. Science *330*, 831–835.

Yano, J.M., Yu, K., Donaldson, G.P., Shastri, G.G., Ann, P., Ma, L., Nagler, C.R., Ismagilov, R.F., Mazmanian, S.K., and Hsiao, E.Y. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell *161*, 264–276.

Zimmermann, M., Zimmermann-Kogadeeva, M., Wegmann, R., and Goodman, A.L. (2019a). Mapping human microbiome drug metabolism by gut bacteria and their genes. Nature *570*, 462–467.

Zimmermann, M., Zimmermann-Kogadeeva, M., Wegmann, R., and Goodman, A.L. (2019b). Separating host and microbiome contributions to drug pharmacokinetics and toxicity. Science *363*, eaat9931.