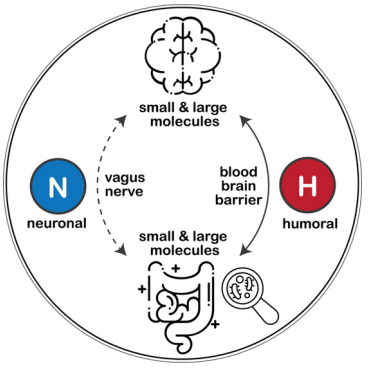
Toward Elucidating the Human Gut Microbiota–Brain Axis: Molecules, Biochemistry, and Implications for Health and Diseases

Yunjia Lai, Radhika Dhingra, Zhenfa Zhang, Louise M. Ball, Mark J. Zylka, and Kun Lu*

ABSTRACT: In recent years, a substantial amount of data have supported an active role of gut microbiota in mediating mammalian brain function and health. Mining gut microbiota and their metabolites for neuroprotection is enticing but requires that the fundamental biochemical details underlying such microbiota–brain crosstalk be deciphered. While a neuronal gut–brain axis (through the vagus nerve) is not disputable, accumulating studies also point to a humoral route (via blood/lymphatic circulation) by which innumerable microbial molecular cues translocate from local gut epithelia to circulation with potentials to further cross the blood–brain barrier and reach the brain. In this Perspective, we review a realm of gut microbial molecules to evaluate their fate, function, and neuroactivities *in vivo* as mediated by microbiota. We turn to seminal studies of neurophysiology and neurologic disease models for the elucidation of biochemical pathways that link microbiota to gut–brain signaling. In addition, we discuss opportunities and challenges for advancing the microbiota–brain axis field while calling for high-throughput discovery of microbial molecules and studies for resolving the interspecies, interorgan, and interclass interaction among these neuroactive microbial molecules.



1. MICROBIOTA, GUT–BRAIN AXIS, AND MOLECULES

The advancement of next-generation sequencing (NGS) technologies over the past decade has led to explosive growth in the knowledge of the trillions of microorganisms colonizing our gastrointestinal (GI) tract, collectively known as gut microbiota.[1] These resident microbes have been demonstrated to mediate important physiological processes in mammalian host bodies, ranging from energy metabolism,[2] vitamin biosynthesis,[3] and immune cell development[4] to hormonal regulation[5] and epithelial homeostasis,[6] and are appealing in new therapeutic venues such as countermeasure against radiation-induced injuries.[7] In turn, when perturbed or in an imbalanced state (i.e., dysbiotic), gut microbial communities have been linked to a long list of diseases and conditions, including metabolic syndrome (e.g., obesity and type II diabetes), autoimmune conditions (e.g., inflammatory bowel disease), and cancer (e.g., colon cancer). Notably, emerging studies on neurologic pathologies such as autism,[8−10] Alzheimer's disease,[11−13] amyotrophic lateral sclerosis (ALS),[14] and multiple sclerosis[15] collectively support the idea that microbiota also actively interacts with the mammalian brain. This raises interesting questions about gut microbiota's neuroactivity and the associated effects on the gut–brain axis, i.e., the bidirectional communication between the central nervous system (CNS, the "first brain") and the enteric nervous system (ENS, the "second brain").[16]

Accumulating research, mostly *in vivo* model-based, has explored microbiota's roles in the neuronal, immune, and endocrine aspects of the two-way gut–brain crosstalk.[17−19] Sudo and colleagues first reported that commensal microbiota in mice perturbed stress responses through the hypothalamic–pituitary–adrenal (HPA) axis.[20] Compared with their conventional wild-type counterparts, pathophenotypes observed in germ-free (GF) mice such as heightened stress responses and low brain-derived neurotrophic factor (BDNF) levels in cortex and hippocampus were substantially restored through select microbial reconstitution during a critical developmental window. This indicated a causal but conditional role of microbiota in stress response regulation. Likewise, mice regularly fed *Lactobacillus rhamnosus* exhibited improved emotional behavior likely owing to altered neurotransmission in the central GABAergic system mediated through the vagus nerve.[21] In a seminal work probing the effects of gut bacteria on autism spectrum disorder (ASD) pathogenesis,[8] maternal immune activation (MIA) during pregnancy successfully induced murine offspring ASD-mimicking defects in the GI barrier and behaviors. These defects were dramatically restored by inoculating human *Bacteroidetes fragilis*, suggesting for autism a molecular mechanism through effects of gut bacterial

Special Issue: Microbiome

Received: October 1, 2021
Revised: December 6, 2021

products on the host metabolome. More recently, fecal microbiota transplantation (FMT) from young mice (3−4 months old) reversed aging-associated cognitive behavioral deficits (e.g., long-term spatial memory) in aged recipient mice (19−20 months old) while offsetting immunological, transcriptome, and metabolome differences, providing fundamental evidence for using FMT as a therapeutic approach toward healthy aging.[22] Conversely, improved neurogenesis and prolongevity signaling were observed in FMT on germ-free mouse models, as well (from old to young).[23] All of these pioneering studies indicate an active role of gut microbiota in brain development and function, opening doors of microbiome-based therapies for improved neurologic/mental health outcomes.

While the idea of targeting microbes for neuroprotection is enticing, controversies regarding how a microbiota−gut−brain axis can be defined and determined for human populations remain. Not until recently, however, has the first population-scale evidence linking microbiota to mental health been published,[24,25] moving beyond the limitations of mainstream evidence mostly derived from animal models. By correlating fecal metagenomic features with quality of life (QoL) and depression indicators for the large Flemish Gut Flora Project (FGFP) cohort, Valles-Colomer and colleagues associated butyrate-producing *Faecalibacterium* and *Coprococcus* with higher QoL scores while identifying considerably lower levels of *Dialister* and *Coprococcus* spp. in patients diagnosed with depression. Gut−brain module analyses singled out the microbial synthetic potential of 3,4-dihydroxyphenylacetic acid (DOPAC), a dopamine metabolite, as being positively correlated with QoL scores alongside altered γ-aminobutyric acid (GABA) pathways in depression. In another cohort study, metagenome-wide association of gut microbial features in schizophrenia patients identified microbial short-chain fatty acid (SCFA) synthesis, tryptophan metabolism, and synthesis/degradation of neurotransmitters as contributors to schizophrenia pathogenesis.[26] Further transplantation of schizophrenia fecal-enriched *Streptococcus vestibularis* in mice induced deficits in social behavior and perturbation of peripheral neurotransmitter profiles, while microbiota-related metabolite trajectories were examined across the perinatal period associated with mental health outcomes, overall revealing substantial temporal variation in tryptophan and bile acid metabolism with marked interindividual variability.[27] Although these human cohort studies are by nature descriptive, they strengthen the notion that the gut microbiota harbors neuroactive potentials that can be harnessed for improved gut−brain signaling, physiologies, and neural health outcomes.

In the targeting of microbiota for neuroprotection, the biochemical underpinnings linking microbiota to the gut−brain axis remain elusive.[28,29] While the neuronal pathway (through the vagus nerve connecting the CNS to the ENS) is not disputable, a growing body of evidence also points to a humoral route (via blood and lymphatic circulation)[30] given the massive enzymatic activities and high turnover rates of microbiota-driven metabolism, especially in neurotransmitter production.[19,31] Due to the relatively small sizes and/or ease of interorgan transportation, innumerable microbial molecular cues may likely translocate from local gut epithelia to the circulating blood and further cross the blood−brain barrier (BBB) to reach the brain while harboring potent signaling/regulatory potentials.[32,33] A growing number of studies showed that gut microbe-related molecules modulate host homeostasis,[34,35] alter barrier permeability,[36] and induce neuroimmune responses,[37] with multifaceted functional roles, for example, as neurotransmitters,[38] as reactive oxygen species (ROS) scavengers,[39] and/or as ligands for activating immune signal transductions.[40]

In this Perspective, we conduct a comprehensive and up-to-date review of the reported molecules of gut microbial sources, including both metabolites and peptides, to evaluate their biochemical fate and functional roles in host−microbe interaction. Importantly, we turn to seminal models of neurophysiology, neurologic diseases, and/or mental disorders to elucidate specific biochemical pathways of the microbiota−gut−brain axis in health and disease. Lastly, we discuss the implications of existing studies, especially the major challenges and opportunities for advancing microbiota−brain research for health sciences spanning the biomedical, nutrition, and public health arenas.

2. SMALL MOLECULES

2.1. Short-Chain Fatty Acids (SCFAs). SCFAs are saturated fatty acids consisting of one to six carbon atoms. In mammalian colon, acetate, propionate, and butyrate are produced in large quantities through microbial fermentation of indigestible dietary fibers, comprising >95% of total SCFAs with a typical molar ratio of 6:2:2.[41] The specific biosynthesis, uptake, and distribution details *in vivo* have been summarized recently.[42−44] Acetate can be broadly transformed by gut bacteria from acetyl-CoA, which is derived from pyruvate, a key intermediate of the glycolysis−citric acid cycle.[41] More substrate-specific and species-conserved, propionate is synthesized through multistep reduction of lactate or succinate or depends on substrates of deoxyhexose sugars (e.g., fucose and rhamnose),[43] whereas butyrate can be formed either from downstream acetyl-CoA products such as butyryl-CoA and butyryl-phosphate, from lactate and acetate by certain gut bacterial species (e.g., *Eubacterium hallii* and *Anaerostipes caccae*), or from protein through the lysine pathway, as indicated by emerging metagenomics data.[45] The concentration profile of SCFAs varies significantly along the GI tract but typically peaks at cecum and proximal colon, followed by a decline toward distal colon.[42] Such a luminal SCFA decline can be partially explained by the transport of acetate and propionate into the circulating blood and local consumption of butyrate by colonocytes.[42]

SCFAs have been intensely studied as key bacterial metabolites conferring a multitude of benefits for host health, with confirmed SCFA producers such as *Bacteroides* spp., *Clostridium* spp., and *Streptococcus* spp.[42] Because SCFAs act as an important energy source with signaling activities, a large proportion of the existing studies targeted SCFAs for linking microbiota to metabolic syndromes such as diabetes and obesity. Interestingly, although SCFAs manifest metabolic benefits like improved glucose tolerance[46] and increased thermogenesis,[47] fecal SCFA levels in obese patients are higher than in the lean controls, which warrants elucidation in the future.[48] SCFAs also contribute to gut integrity and immune homeostasis; inside gut lumen, SCFAs modulate the pH, balance redox equivalent production, and fortify the gut lining.[41,43,49] In immune regulation, SCFAs activate G protein-coupled receptors (GPRs), inhibit histone deacetylases (HDACs),[42] control regulatory T cell (T_{regs}) expansion,[50] and regulate local synthesis and release of key endocrine

agents, including glucagon-like peptide 1 (GLP-1)[41] and serotonin.[51]

Mounting studies show profound effects of gut microbial SCFAs on host brain function and/or CNS-mediated pathogenesis through, for example, regulating host neurodevelopment (e.g., microglial maturation),[52] modulating BBB permeability,[53] and acting on the ENS for appetite control.[54] Erny and colleagues reported that eradicating a complex host microbiota, either constitutionally (as found in GF mice) or in an induced fashion (e.g., by antibiotics, or abx), led to severe defects in the density, morphology, and maturation of microglia, the resident macrophages in mammalian brain.[52] In turn, a four-week administration of SCFAs (cocktail mix of acetate, propionate, and butyrate) markedly rectified the microglial defects in GF mice. Interestingly, while the *Ffar2*$^{-/-}$ mice (i.e., double knockout of genes encoding free fatty acid receptor 2) exhibited substantial microglial compromises, the FFAR2 protein (also known as GPR43) was not detected in CNS tissues, leaving how SCFAs communicated with the CNS incompletely elucidated. In another work employing mouse models of Parkinson's disease (PD), Sampson et al. identified gut microbiota as a causal contributor to the hallmark motor and GI dysfunction of PD.[55] One mechanism established is postnatal gut−brain signaling by microbial SCFAs to mediate neuroinflammatory responses and aggregation of α-synuclein (αSyn) protein. In addition, Perry and colleagues identified acetate as a microbiome−brain−β-cell axis mediator for promoting metabolic syndromes.[56] Using whole-body turnover rate determination and stable-isotope tracer analysis *in vivo*, the authors observed in high-fat diet (HFD)-fed rats a marked increase in the rate of acetate turnover, for which the gut microbiome was confirmed as arguably the main source. Strikingly, such surging acetate levels promoted glucose-stimulated insulin secretion (GSIS) via activating the parasympathetic nervous system, resulting in hyperphagia, obesity, and related sequelae, all of which were prevented by severing the vagus nerve. In a new work, gut bacteria-derived acetate was discovered to also drive microglial maturation and functions during neurodegeneration, specifically the microglial phagocytosis of amyloid-β and disease progression in a mouse model of AD.[13] Beyond these seminal studies, research also showed that SCFAs modulate BBB,[57] regulate satiety,[41] and interact extensively with the ENS, where local SCFA receptor GPR41 is strongly expressed.[42,58]

2.2. Bile Acids (BAs). BAs are hepatically synthesized steroid acids (*de novo*, from cholesterol) that are subsequently conjugated to taurine, glycine, phenylalanine, tyrosine, and leucine (collectively termed primary BAs).[59,60] Upon food intake, primary BAs are discharged from the gallbladder into the gut lumen, where they emulsify dietary fats and experience massive microbial transformation into bioactive products known as secondary BAs.[59] With amphipathic functional groups, BAs exhibit detergent-like properties that facilitate lipid uptake, transport, and metabolism, with the enterohepatic circulatory pool cycle occurring at a frequency that depends on dietary patterns. Interestingly, more recent work revealed BAs as signaling molecules, as well. For example, BAs activate the nuclear receptor farnesoid X receptor (FXR) and Takeda G protein-coupled bile acid receptor (TGR5), both of which, although working seemingly independently, have been shown to be potent regulators of BA synthesis, energy metabolism, and immune cell homeostasis.[61−63]

Understanding gut microbiota's control over BA synthesis and signaling will benefit the fundamental design of therapeutic solutions for diseases ranging from diabetes[64] and non-alcoholic fatty liver disease (NAFLD)[65,66] to colon cancer.[67] On one hand, BAs curtail gut bacterial growth toward a more balanced ecological state that contributes to gut epithelial homeostasis. On the other hand, gut bacteria harbor a versatile enzymatic toolkit (e.g., glucuronidase and dehydroxylase) that drives biotransformation of primary BAs into secondary BAs that typically embrace increased chemical structural diversity and signaling potency.[68,69] Targeted profiling of BAs in GF-treated, abx-treated, and conventionally raised rats showed that the absence of microbes (as found in both GF- and abx-treated mice) led to less diverse BA profiles dominated by taurine-conjugated BAs at extrahepatic sites of kidney, heart, and blood relative to those in conventional counterparts.[70] In addition, Sayin et al. identified for conventional wild-type C57BL/6 mice decreased enterohepatic levels of muricholic acid (MCA) but not cholic acid (CA) compared with those of GF individuals.[71] Using *Fxr*$^{-/-}$ murine strains, the study demonstrated gut microbiota's control over the expression of ileum fibroblast growth factor 15 (FGF15) and hepatic 7α-cholesterol hydrolase in a farnesoid X receptor (FXR)-dependent manner, specifically through reducing tauro-β-muricholic acid (TβMCA), a natural FXR antagonist. Remarkably, Quinn and colleagues recently discovered new BA conjugates (in addition to tauro and glyco species) with massive chemical impacts from microbiota, improving our fundamental understanding of the complex and diverse bile acid chemistry.[60] Comparative microbial (16s rRNA amplicon sequencing) and metabolite profiling (molecular networking-based annotation) across 29 organs (and/or matrices) of GF versus colonized mice identified a unique panoply of contrasting chemical signatures in the GI tract owing to microbiota, later structurally revealed as amide BA conjugates, including phenylalanocholate, tyrosocholate, and leucocholate, that have never before been reported; with much human health relevance, their functional roles related to FXR signaling were further confirmed *in vitro* and *in vivo*.

To date, a growing number of studies have been reported linking BAs to the evolving definition of the microbiota−gut−brain axis. Liu and colleagues showed that BAs induced antidiabetic effects through signaling FGF receptor 1 (FGFR1) in hypothalamic agouti-related protein (AgRP)-producing neurons.[72] In *ob/ob* obese mice, oral gavage of taurocholic acid markedly improved glucose tolerance by upregulating the level of ileum expression of FGF15 (murine counterpart of human FGF19), likely through an FXR-specific pathway. Other studies suggest that bacterially derived BAs trigger a gut−liver−brain axis for satiety control,[73] for improved glucose metabolism (as found for bariatric surgery),[74,75] and to remedy alcohol-associated liver injuries even in cases of cirrhosis and alcoholic hepatitis.[76]

BAs are linked to altered brain function and behaviors, as well. One work employing rodent ASD models associated decreased abundances of gut bacterial species involved in BA metabolism (e.g., *Bifidobacterium* and *Blautia* spp.) with increased GI dysfunctional and autism-like behavioral scores.[77] Another work reported that a gut-based bariatric surgery led to larger bile acid pools, an attenuated cocaine-induced increase in dopamine levels in nucleus accumbens, and reduced addiction-related behaviors; using knockout mouse models, the study further identified TGR5 and bile acid signaling as the

key mediator.[78] In a recent study, a humoral route via which the circulating BAs reach the brain and control the satiety was confirmed, where BA administration, either peripheral or central, led to an anorexigenic effect in a TGR5-specific manner.[79]

Large human cohort studies (n > 1400) of Alzheimer's disease (AD) associated altered serum BA profiles with markers of cognitive decline and AD pathophysiology, supporting the hypothesis that circulating BAs contribute to AD pathogenesis in which gut microbiota plays a role.[80,81] Notably, significantly lower levels of circulating CA (a primary BA) and higher levels of deoxycholic acid (or DCA, a secondary BA converted from CA by bacteria), alongside their glycine and/or taurine conjugates, were observed in AD patients compared to their age-matched normal controls. A strong positive association was identified and validated among the surging DCA:CA ratios and markers of cognitive impairment, indicating gut bacterial 7α-dehydroxylation of CA as one mechanistic route of AD pathogenesis. For specific modes of action, questions remain about whether BAs act locally on TGR5-expressing ENS to signal the CNS[61] or originating from peripheral circulation, they cross the BBB (in unconjugated nonpolar forms), reach the CNS, and act *in situ*, as indicated by previous data.[82−86] In addition, the functional roles of BAs in AD pathogenesis exhibited gender specificity, as revealed in a targeted metabolomics study examining 28 BAs in enterohepatic circulation of AD transgenic (PP/PS1 induced) and wild-type colonized mice. Compared with conventional controls, female AD transgenic mice had decreased levels of primary BAs (taurine-conjugated) and increased levels of secondary BAs in plasma and liver, while their male counterparts exhibited the opposite trend.[87]

2.3. Tryptophan Catabolites. L-Tryptophan (Trp) is an essential aromatic amino acid exclusively obtained from the diet via eggs, fish, and milk, with a required daily intake of 3.5 mg/kg of body weight.[88] Trp is actively involved in protein biosynthesis and cell growth while serving as a substrate in producing metabolites that are crucial for host physiological processes spanning immune cell homeostasis, energy balance, and neurotransmission.[88] In mammals, Trp is transported throughout the body (except across the BBB) in albumin-bound forms by large neutral amino acid transporters (LATs).[89] Trp catabolism (only in free form) occurs mostly inside the GI tract via three distinct pathways, namely, the kynurenine pathway (KP), the serotonin pathway, and production of indoles.[90] In the human body, >95% of Trp is metabolized through KP, generating a realm of kynurenine (Kyn) derivatives with wide-ranging neuroactivities, including the end product nicotinamide adenine dinucleotide (NAD^+), a cofactor essential for cellular energy metabolism.[88,91] The Trp−Kyn pathway has been detailed from the perspective of both evolution and drug development.[88,92] Smaller portions of Trp are converted into serotonin (5-HT) and other downstream neurotransmitters such as *N*-acetylserotonin (NAS), melatonin, and 5-hydroxyindole acetate (5-HIAA) that are potentially implicated in microbe−brain crosstalk.[93,94] In addition, gut microbiota transforms Trp into indole and/or indolyl derivatives, among which many have been identified as key regulators of inflammation and mucosal homeostasis.[95,96]

The crucial role of all three Trp catabolic fluxes in orchestrating the gut−brain signaling has been increasingly identified, particularly in cases associating gut microbiota and neuropathologies.[97,98] The notion of a microbiota−brain axis has been recently buttressed by the aforementioned FGFP cohort study,[25] in which gut−brain module (GBM) analyses identified the presence of animal-like GBM (5-HT synthesis I) rather than the plant-like GBM (5-HT synthesis II) in roughly 20% of gut-associated genomes from the Integrated Microbial Genomes (IMG); microbial genera, including *Akkermansia* and *Alistipes*, were discovered as potential 5-HT producers. Such findings may open doors for targeting gut microbiota-regulated Trp pathways for improved mental health outcomes.

2.3.1. Serotonin. Perhaps the best known biochemical details of microbiota−Trp interaction revolve around serotonin [or 5-hydroxytryptamine (5-HT)], a monoamine neurotransmitter that regulates mood control, appetite, and cognitive behaviors in brain as well as motility[99] and hemostasis[100] in the GI tract. Strikingly, >90% of 5-HT is synthesized *de novo* by gut endocrine cells such as enterochromaffin cells (ECs) and myenteric neurons, where commensal microbes are actively involved. Novel signaling cascades by which the microbiota acts on local 5-HT biosynthesis were recently reported; one is through direct signaling of colonic ECs to promote gene expression of tryptophan hydrolase 1 (TPH1).[101] The gut microbiota was also discovered to constantly regulate ENS maturation even until adulthood via an enteric 5-HT network.[102] Note that reportedly gut-derived 5-HT cannot cross the BBB, and 5-HT levels in brain would thus solely depend on its CNS synthesis *in situ*;[28] how microbe-mediated 5-HT metabolism in the ENS (and the extended circulatory or peripheral systems) mediates CNS activities and leads to neuro-related changes constitutes a critical direction of future research.

2.3.2. Kynurenines. Kynurenines, including Kyn and the downstream catabolites, are increasingly recognized to regulate brain health. In CNS, 40% of the Kyn pool is synthesized *de novo*, whereas the remaining 60% comes from peripheral sites through crossing the BBB. For the Trp−Kyn pathway occurring in peripheral tissues where enzyme levels are much higher than in brain, the first step is the rate-limiting one. Both extrahepatic indoleamine 2,3-dioxygenases (IDOs) and hepatic tryptophan 2,3-dioxygenase (TDO) catalyze the conversion from Trp to *N*-formylkynurenine, the immediate precursor of Kyn.[103] Kyn metabolism in brain yields disparate metabolite sets with cell-specific effects. One is microglial generation of 3-hydroxykynurenine (3-HK) and its immediate derivative, quinolinic acid (Quin); 3-HK is linked to oxidative stress and apoptosis inside the CNS, while Quin exhibits neuronal excitotoxicity through activating the *N*-methyl-D-aspartate receptor (NMDARs).[104−106] The other is kynurenic acid (Kyna), a CNS astrocyte-producing Kyn derivative that exerts neuroprotective effects by serving as an antagonist to both NMDARs and α7-nicotinic acetylcholine receptors (α7 nAChRs)[107] and as an agonist for the aryl hydrocarbon receptor (AhR),[108] a master immune regulator and an important transcription factor in xenobiotic metabolism. Measuring Quin:Kyna ratios in the CNS, therefore, will help monitor the status of CNS excitation and neuroinflammation.[88,109] Overall, accumulation of Kyn in the CNS due to peripheral inflammation has been generally associated with mental disorders such as depression and schizophrenia;[110] it would thus be beneficial to dissect the role of gut microbiota in controlling peripheral Kyn availability and to systematically identify microbial Trp metabolites that can be transported across the BBB alongside the associated transporters and cellular receptors.

2.3.3. Indoles. Bacterial Trp products, notably indole and its indolyl derivatives, have been identified as potent regulators that sustain mucosal homeostasis while eliciting proper immune responses.[111] One striking example is indole-3-propionic acid (IPA), an indolyl metabolite exclusively from Clostridiales family bacteria such as *Clostridium sporogenes*.[34] IPA plays an active role of the ROS scavenger itself (i.e., an antioxidant), activates the AhR receptor to promote the release of anti-inflammatory cytokines [e.g., interleukin-10 (IL-10)], and suppresses proinflammatory cytokines such as tumor necrosis factor-α (TNF-α).[112] Likewise, indole-3-acrylic acid (IAcrA), produced by *Peptostreptococcus russellii* (a mucin user), is an AhR agonist leading to suppressed inflammation. Indole-3-acrylic acid (IAcrA) also binds to the pregnane X receptor (PXR) to promote gene expression of *muc2* that encodes mucin 2 to enhance the integrity of the intestinal epithelial barrier (IEB).[113]

Reported evidence has linked microbial indoles to CNS function and health. One *in vivo* study of multiple sclerosis (MS) demonstrated that bacterially Trp-derived indole, indole 3-sulfate (I3S), IPA, and indole 3-carboxaldehyde (I3A), can modulate CNS inflammation directly by activating the AhR receptor expressed in astrocytes.[114] Another study of the human cohort correlated indole, indole-3-acetic acid, and skatole levels with quantitative readouts of functional and anatomical connectivity for the extended central reward network that incorporates amygdala, nucleus accumbens, and anterior insula. Association between these indoles and body mass index (BMI), food addiction, and anxiety symptoms was also discovered.[115] Note that microbiota-derived indoles can easily enter mammalian circulation and reshape host blood metabolomes.[30,34,111] Further research should thus be conducted to identify and track peripheral indole species that can reach the brain and to examine how they mediate CNS-resident immune homeostasis and neurological functions.

2.4. Neurotransmitters. Neurotransmitters are presynaptic compounds that evoke postsynaptic electrical signaling (through binding to receptors *in situ*) to fulfill neurophysiological functions, typically with a fast complete cycle of synthesis, release, and reuptake.[116] In the human GI tract, multiple neurotransmitters, including GABA, 5-HT, and histamine, are synthesized in large quantities by gut bacteria. This has led to an increasing number of inquiries about the involvement of neurotransmitters in microbe−brain crosstalk and regulation of local gut microbial growth and ecology.[19,117,118] Microbial endocrinology theories are continuously developing for neurotransmitters that embrace evolutionarily shared synthetic pathways, receptor families, and neuroendocrine effects among mammals and microbes.[119−121] Such interkingdom crosstalk in the language of neurotransmitters was supported by the landmark FGFP cohort study.[25] The results associated perturbed GABA synthetic pathways, 5-HT metabolism, and dopamine derivatives with quality of life (QoL) proxies and depression status. Here, we focus on amino acids (e.g., glutamate, GABA, and 5-HT) and biogenic amines (e.g., dopamine, norepinephrine, and epinephrine).

2.4.1. Glutamate and γ-Aminobutyric Acid. L-Glutamate (Glu) and γ-aminobutyric acid (GABA) are the major excitatory and inhibitory neurotransmitters, respectively, in the human CNS, and interestingly, they are biochemically interconvertible via the two-way (glutamine)Glu−GABA cycle.[116,122] Both glutamatergic and GABAergic loops incorporate the fate of Glu/GABA, neurotransmission through activating widespread receptors in the host, and inactivation by high-affinity transport systems in glial cells (mainly astrocytes), with the machineries detailed elsewhere.[123] For Glu−GABA metabolism, it is generally agreed that Glu cannot pass the BBB[124,125] and, therefore, CNS Glu originates from only *in situ* synthesis, relying on glutamine (Gln) as the precursor or through transamination of 2-oxoglutarate from the citric acid cycle.[123,126] On the contrary, evidence supported the idea that GABA could cross the BBB.[127,128] In the CNS, GABA can be derived from glycolysis or from Glu as catalyzed by L-glutamic acid decarboxylase (GAD), an enzyme almost exclusively present in GABAergic neurons. In parallel, innumerable bacterial strains have been discovered to be producers of Glu (e.g., *Bifidobacterium* spp., *Lactobacillus* spp., and *Corynebacterium glutamycum*) and GABA (e.g., *Lactobacillus* spp., *Escherichia coli*, and *Pseudomonas* spp.) for functional purposes, including interspecies interaction.[123] Studies revealed that both Glu and GABA serve as energy substrates for gut microbes, balance luminal pH, modulate pathogen virulence, regulate gut motility, etc.[117,123] In this light, the microbe−brain axis through the glutamatergic and/or GABAergic system may be a viable pathway to explore.

Studies linking gut bacteria-derived Glu and GABA to neurological health phenotypes are emerging. In a landmark study,[21] the lactic acid bacterial strain *L. rhamnosus* has been demonstrated to exert a direct effect on murine CNS GABA receptor expression while conferring anxiolytic benefits in mice, as shown in elevated plus maze (EPM) behavioral test and stress hormone analyses. Through vagomization trials, the authors further identified the vagus nerve as a constitutive pathway of communication between gut-resident microbes and brain. More recently, Pokusaeva and colleagues showed in a rat model that oral administration of *Bifidobacterium breve* NCIMB8807 pESHgadB, an engineered GABA-producing strain with overexpressed *GadB* genes encoding the GAB protein, led to reduced sensitivity to visceral pain compared to wild-type strains.[38] In another work, Zheng and colleagues combined fecal transplant trials and revealed that gut microbiota of schizophrenia patients could modulate the Gln−Glu−GABA cycle and induced schizophrenia-relevant behaviors in mice.[129]

2.4.2. Catecholamines: Dopamine, Norepinephrine, and Epinephrine. Catecholamines, including dopamine, norepinephrine, and epinephrine, are biogenic amine neurotransmitters that carry crucial roles in a wide range of neurophysiological and behavioral processes, spanning CNS homeostasis, attention, emotional control, and coordination of body movement.[116,130] In mammalian brain, dopamine is produced in the cytoplasm of presynaptic terminals through decarboxylation of its immediate precursor, L-3,4-dihydroxyphenylalanine (L-DOPA), that is derived from tyrosine (Tyr) as a rate-limiting step. In downstream reactions, dopamine serves as a substrate for producing norepinephrine and epinephrine.[116] Although the three compounds are biochemically interconvertible, they carry distinct activities and functions in the CNS. More specifically, dopamine, mainly located in corpus striatum (an area for motor control), carries essential roles in motivation, reinforcement, reward, and hedonistic regulation.[131] Many substances of abuse (e.g., cocaine and amphetamine) work by altering dopaminergic synapses in the CNS. Moreover, drug design for psychotherapeutic use typically mimics dopamine's structure to fulfill antianxiety,

antidepressant, and antipsychotic benefits. Norepinephrine is known to regulate attention, sleep/wakefulness, and feeding behavior, whereas epinephrine, at much lower CNS levels, may have an impact on memory and learning.[116]

The microorganisms have long been recognized as catecholamine producers. For example, *Bacillus*, *Serratia*, and *Proteus* spp. synthesize dopamine, while *Escherichia*, *Bacillus*, and *Saccharomyces* spp. are known to produce norepinephrine.[118,119,132] Although how gut commensal bacteria contribute to host catecholamine metabolism remains to be revealed, accumulating data conferred such insights.[133−136] Heijtz et al. discovered contrasting neurophenotypes between GF and specific pathogen-free (SPF) conventional mice based on behavioral test scores, neurochemical concentration profiles, and patterns of gene expression. Increased turnover ratios of dopamine and norepinephrine were found in GF mice, suggesting a role of microbiota.[134] In addition, Kiraly and colleagues revealed in a mouse model that antibiotic knockdown of gut microbiota, without affecting cocaine metabolism and behavior-modulating stress hormones, resulted in altered cocaine-mediated behaviors, as characterized by heightened sensitivity to cocaine reward in conjunction with changes to the dopaminergic system such as increased activity of CNS D1 dopamine receptor *Drd1*.[135] More recently, human cohort studies associated gut microbial compositions and functional genes with altered dopaminergic systems in disease models such as depression and PD.[25,137] However, as all three polar catecholamines cannot cross the BBB,[116] how bacterially mediated catecholamine metabolism interacts with gut−brain crosstalk remains elusive and warrants elucidation.

2.4.3. Other Small-Molecule Neurotransmitters. Aside from 5-HT, glutamate/GABA, and catecholamines, many other neurotransmitters of confirmed microbial sources may also mediate gut−brain signaling and alter paths of associated psychopathologies, although the current evidence is only emerging or at best suggestive. These span histamine, trace amines (*β*-phenylethylamine, tyramine, and tryptamine), glycine, acetylcholine (ACh), serine, and taurine. For instance, histamine is an organic nitrogenous mediator of inflammatory responses and itching; histamine contributes to tissue swelling during inflammation by increasing the permeability of capillaries to white blood cells and proteins. One study showed the probiotic strain *Lactobacillus reuteri* (with histidine decarboxylase) converts histidine to histamine, which suppresses TNF through modulation of protein kinase (PKA) and extracellular signal-regulated kinase (ERK) signaling.[138] Likewise, trace amines tryptamine and *β*-phenylethylamine are known to play significant roles in appetite control, attention, and emotional processing. A range of lactic acid bacteria have been confirmed as their synthesizers, with *Lactobacillus bulgaricus* and *C. sporogenes* for producing tryptamine and *Enterococcus faecalis* and *Leuconostoc* strains for producing both *β*-phenylethylamine and tyramine.[139,140] In a recent work, two gut bacteria-derived carnitine analogues, 3-methyl-4-(trimethylammonio)butanoate and 4-(trimethylammonio)pentanoate, were newly identified as mediators of gut−brain communication, specifically through inhibition of carnitine-mediated fatty acid oxidation in brain white matter.[141] Future studies should examine neurotransmitters for which kinetics of microbial synthesis in gut are better modeled, the gut microbial proportion to the CNS levels is differentiated, and their functional roles in modulating gut−brain signaling are delineated.

3. LARGE MOLECULES

3.1. Microorganism-Associated Molecular Patterns. On both molecular and ecological levels, microorganisms are in constant flux of biochemical signaling for intra- and/or interspecies communication, inhabitant assessment, trigger of immune responses, formation of symbiotic biofilm, etc.[142,143] At the center are the microorganism-associated molecular patterns (MAMPs),[144] highly class-specific and evolutionally conserved microbial protein or peptide molecular motifs through which microbial species identify one another. As evidenced, MAMPs, of which many are pathogens to mammals, can translocate from the gut to the remote peripheral blood system to induce immune responses, raising the possibility that they are involved in the microbiome−gut−brain axis,[145] the two most notable examples being lipopolysaccharides (LPS) and bacterial peptidoglycans (PNGs).

3.1.1. Lipopolysaccharides. Lipopolysaccharide (LPS) (or endotoxin) is a major component of Gram-negative gut bacteria and can translocate from the gut lumen to the systemic circulation through leaky mucosal linings. Once sensed by pattern recognition receptors (PRRs) [e.g., Toll-like receptor 4 (TLR4)] of the host innate immune system, cytokines are produced that can either signal directly to the CNS or excite vagal and spinal afferent neurons, given that PRRs of LPS (e.g., TLRs) are prevalently expressed in GI epithelial cells, ENS neurons, and the rest anatomical makeup of the gut−brain axis.[146] There have been multiple studies linking intestinal microbial LPS to brain health. Wu and colleagues observed significantly higher circulating and brain levels of LPS in aged mice compared with young controls while associating age with gut dysbiosis and markers of neuroinflammation.[147] Maes and colleagues reported for patients with both chronic fatigue syndrome[148] and chronic depression[149] increased circulating immunoglobulin levels (e.g., IgA and IgM) in response to propagated gut bacterial LPS, suggesting a role for LPS in the microbe−brain interplay.

In parallel, administration of LPS, through injection or oral gavage, can lead to exacerbated neurological pathophenotypes in mice such as depression-mimicking behaviors[150] and cognitive impairment.[151] These are collectively demonstrated for LPS as potent molecular cues of microbiota to influence host brain function and behaviors.

3.1.2. Peptidoglycans. Peptidoglycans (PGNs) constitute another type of (Gram-positive) bacterial peptide with effects on brain function. A seminal study by Arentsen and colleagues reported for GF mice (compared with normal mice) extremely low or below-detection-limit serum PGN levels using ultrasensitive mass spectrometry platforms, suggesting gut microbiota as a major source for peripheral PGNs.[152] Microbial PGNs, once relocated to circulating blood (including under normal physiological conditions) and recognized by cytosolic nucleotide-binding and leucine-rich repeat-containing receptors (NLRs) (the major PRRs of PGNs), trigger downstream immune responses and regulate CNS function. Recent evidence supports such a link between microbial PGNs and CNS health.[153] On one hand, the NLRs play an active role in shaping and controlling gut microbiota, with notable examples of NLRP6 and NLRP12 demonstrated to regulate intestinal inflammation, gut microbial composition, and metabolism.[154] Pusceddu and colleagues also revealed a novel role of NLRs in gut−brain signaling.[155] The authors observed for knockout mouse models (NodDKO) deficient in both *Nod1* and *Nod2*

exacerbated stress-induced behaviors, an impaired cerebral serotonergic system, a decline in hippocampal neurogenesis, and increased GI permeability. In turn, administration of fluoxetine, a selective 5-HT reuptake inhibitor, corrected these behavioral deficits while restoring 5-HT signaling. As for PGNs, the MIA ASD model has been established as maternal immune activation itself, through infecting pregnant mice with proinflammatory MAMPs (e.g., PGNs), which induces ASD-like behaviors in mouse offspring. Such effects of PGNs at the maternal−fetal interface suggest a critical role of maternal microbiota in shaping fetal brain health. This was supported by the latest studies examining the placental mobility of the PGN−teichoic acid complex (a cell wall component of pathogen *Streptococcus pneumoniae*) in parallel with fetal outcomes of neuro-proliferation and cognitive development;[156] the details are reviewed elsewhere with critical questions proposed.[157]

3.2. Peptides of Neurodegenerative Hallmarks: α-Synuclein and β-Amyloid. Much interest has been stimulated in researching the relationship between gut microbiota and pathogenesis of neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD). For AD and PD, aggregated CNS levels of β-amyloid (Aβ) and α-synuclein (α-syn), respectively, are the two main associated pathological hallmark proteins.[158,159] A recent work discovered the capability of microbes to produce extracellular amyloid-like proteins that in turn exacerbate in aged rats the pathogenic course of α-syn, indicating multifaceted effects of microbiota on neurodegeneration.[160] Note that α-syn, once in the pathologic state, has a prion-like activity to propagate and translocate through the vagus nerve of the gut−brain axis and leads to impaired CNS physiology and behavioral deficits.[161] This hypothesis was supported by a recent report by Sangjune and colleagues.[162] The authors performed gut injection of α-syn fibrils and discovered that murine endogenous α-syn was converted to a pathologic species that could spread from the gut to the brain, resulting in pathological traits of PD, including degeneration of dopamine neurons and motor (and non-motor) deficits. Strikingly, these traits were prevented via truncal vagotomy or α-syn deficiency (by knockout of the *Snca* gene), supporting the vagus nerve as an essential conduit for the α-syn pathology of PD. This neuronal route was further supported by epidemiological studies (e.g., Danish cohort[163] and Swedish cohort[164]) that aimed to dissect the effects of truncal vagotomy procedures on PD risks. Because the mucosal microbiota is in the vicinity of the ENS, whether and how the gut microbiota contributes to α-syn pathogenesis in PD through the vagus nerve remains to be resolved.[165]

3.3. Neuropeptides in Appetite Control. The GI tract, with tubular ENS tissue lining all over, is home to innumerable neuroendocrine cells as well as their neuropeptide products spanning neuropeptide tyrosine (NPY), calcitonin gene-related peptide (CGRP), peptide YY (PYY), and GLP-1. NPY and CGRP are expressed at all levels throughout the gut−brain axis, while PYY and GLP-1 are exclusively produced in distal ileum and colon by local enteroendocrine L cells. Due to their proximity to the intestinal mucosa where the microbiota resides, a question about whether the gut microbiota interacts with these neuropeptides to further modulate gut−brain signaling arises. One relevant topic of research is gut microbial regulation of mammalian appetite control, focusing on SCFAs as a proof of principle. Through *in vivo* and *in vitro* models, one study reported that colonic microbial propionate stimulated local release of PYY and GLP-1 from L cells in wild-type mice, but not in in *Ffa2*$^{-/-}$ mice deficient in expression of FFA2, a receptor for SCFAs. The results suggest that the gut microbiota harbors great potential to modulate neuropeptide profiles, associated satiety perception, and food intake behaviors.[166]

A recent review article underscores the role of the microbiota in appetite control.[131] GABAergic and glutamatergic neurons, both belonging to the hippocampal arcuate nucleus (ARC) family and closely related to the Glu/GABA cycle, are key regulators of appetite status. GABAergic neurons express the orexigenic NPY and agouti-related protein (AgRP), whereas glutamatergic neurons express pro-opiomelanocortin (POMC), a precursor of α-melanocyte-stimulating hormone (αMSH) and an anorexigenic neuropeptide. Often, the NPY, AgRP, and POMC hormone populations are viewed as the "first-order" neurons in satiety and hunger pathways. Most interestingly, recent studies together suggest a causal role of gut bacteria in influencing host energy metabolism and feed behaviors, with colonic bacterial growth profiles overlapping much with patterns of satiety hormone release (e.g., GLP-1 and PYY) and satiety perception, especially during first 20 min upon meal induction.[131] The author also speculated bacterial metabolites (e.g., quorum-sensing acylhomoserine lactones), if detected in portal circulation, might act directly on hypothalamic neurons.

4. IMPLICATIONS

4.1. Discovering Novel Molecules of the Microbiota. Complex and dynamic in constant metabolic fluxes, the gut microbiota harbors innumerable novel molecules yet to be discovered and mined for neuroactive effects. Identifying those through which our commensal microbiota mediates the CNS function thus is a pressing issue to resolve. One example involves bacterial quorum-sensing molecules *N*-acylhomoserine lactones (AHLs) for interspecies communication regarding local population growth and nutrient use. Previous studies showed an effect of gut-derived AHLs on the function of neurons,[167] but whether and how they may reach humoral circulation has only been recently discussed.[168] In addition, the gut microbiota may be a source of steroid hormones; one example is gut bacterial strain *Clostridium scindens* performing such glucocorticoid-to-androgen conversion.[169] Moreover, some bacterially derived molecules, even at low levels, might be implicated in a wide range of host neurophysiological processes. One example is tetrahydrobiopterin (BH_4) that is a cofactor that is essential for normal CNS dopamine synthesis and function. A recent work confirmed gut-residing *Adlercreutzia equolifaciens* and *Microbacterium schleiferi* as BH_4 producers, leading to the question of whether they contribute to the CNS pool of BH_4.[170] Another example is vitamin B_6, which can be bacterially derived in the gut.[171] Vitamin B_6 is the precursor of pyridoxal phosphate, a cofactor essential for GAD enzymatic conversion of glutamate to GABA. The lack of vitamin B_6 can result in a massive decrease in CNS GABA levels, subsequent loss of synaptic inhibition, and seizures.[116] Also, amines of confirmed bacterial sources, including putrescine, spermidine, spermine, and cadaverine, have been shown to be implicated in the responses of the CNS to stress.[172] It should be noted that the authors have recently published a high-coverage metabolomics study comparing GF and wild-type colonized mice, reporting as many as 701 unique differential metabolites owing to the presence of the micro-

Table 1. Reported Tissue/Organ Specificity and Analytical Detection for Representative Molecules (or turnover) of Potential Microbiota–Brain Links from Select Reviewed Research Articles in This Perspective[a]

molecule (or turnover)	abbreviation	type	group	subgroup	organism	tissue/organs	platform	context	ref
acetate	AA	small molecules	fatty acids	SCFAs	mice and cells	brain, colon, liver, and serum; cell culture (primary glial astrocytes, neurons, and microglia)	GC-MS	Alzheimer's disease	13
taurocholate	TCA	small molecules	bile acids	primary, conjugated	mice	hypothalamus	LC-HRMS	TGR5-specific anorexigenic effects	79
deoxycholate	DCA	small molecules	bile acids	secondary, free	mice	hypothalamus	LC-HRMS	TGR5-specific anorexigenic effects	79
ω-muricholate	ωMCA	small molecules	bile acids	primary, free	mice	hypothalamus	LC-HRMS	TGR5-specific anorexigenic effects	79
5-hydroxyindoleacetate/serotonin	5-HIAA/5-HT	small molecules	aromatic amino acids	tryptophan: serotonin	mice	striatum	LC-EC	germ-free mouse-based comparison	64
dihydroxyphenylacetate/dopamine	DOPAC/DA	small molecules	neurotransmitters	catecholamines	mice	striatum	LC-HRMS	germ-free mouse-based comparison	64
3-methoxy-4-hydroxyphenylglycol/noradrenaline	MHPG/NA	small molecules	neurotransmitters	catecholamines	mice	striatum	LC-HRMS	germ-free mouse-based comparison	64
indoxyl sulfate	IS	small molecules	aromatic amino acids	tryptophan: indoles	mice	feces, serum, and cortical brain tissues	LC-HRMS	germ-free mouse-based comparison	30
4-ethylphenylsulfate	4EPS	small molecules	aromatic amino acids	phenols	mice	feces and serum	GC/LC-MS	autism spectrum disorder	8
p-cresol		small molecules	aromatic amino acids	phenols	mice	feces	GC-MS	autism spectrum disorder	9
nicotinamide	NAM	small molecules	vitamins	vitamin B	humans	serum and CSF	UHPLC-MS/MS, GC-MS	amyotrophic lateral sclerosis	14
γ-aminobutyrate	GABA	small molecules	neurotransmitters	glutamate–glutamine–GABA cycle	mice	hypothalamus	LC-HRMS	aging-related FMT	22
glutamate	Glu	small molecules	neurotransmitters	glutamate–glutamine–GABA cycle	mice		LC-QTOF	schizophrenia-related FMT	129
trimethylamine *N*-oxide	TMAO	small molecules	choline	amine oxides	mice	feces, serum, and cortical brain tissues	LC-HRMS	germ-free mouse-based comparison	30
peptidoglycan	PGN	large molecules	biopolymers	sugar-amino acid polymer	mice	brain tissues (prefrontal cortex, striatum, and cerebellum) and serum	SLP	*Pglyrp2* KO mice	152

[a]Abbreviations: SCFA, short-chain fatty acids; CSF, cerebrospinal fluid; GC, gas chromatography; HRMS, high-resolution mass spectrometry; QTOF, quadrupole time-of-flight; EC, electrochemical (detection); SLP, silkworm larval plasma detection; FMT, fecal microbiota transplant; TGR5, G protein-coupled bile acid receptor-1; *Pglyrp2* KO, peptidoglycan recognition protein 2 knockout.

biota.[30] Presenting a valuable data set for future research probing microbial neuroactive potentials, this study points to not only a need to mind tissue/organ specificity when studying microbiota's effects but also the importance of using novel and powerful analytical platforms, including high-resolution mass spectrometry (HRMS) and integrated cheminformatics, to provide hard chemical evidence when examining the microbiota−brain crosstalk. Here, we therefore present a summary of select metabolites of microbiota−brain links for tissue/organ specificity and analytical detectability (Table 1).

4.2. Unraveling Interclass Interaction. The microbiota−gut−brain axis field is currently undergoing a paradigm shift from asking (i) what microbes are to (ii) what they do and, eventually, (iii) how we could leverage them for health causes. Measures should be taken to go beyond curating gut microbial neurochemicals and dissecting their role (in a reductionist manner) toward gaining an integrated perspective of them together. In other words, it is important to identify the interaction between seemingly distinct compound classes by structures, sources, and biochemical pathways for systems biology-level insights. There is no lack of data supporting such cross-class interaction and/or interspecies communication. Notable examples included interaction between bacterial SCFAs and tryptophan metabolites; for example, *Clostridium* bacterially derived SCFAs have been shown to modulate microbial 5-HT biosynthesis in gut enterochromaffin cells, specifically through promoting gene expression of *Tph1*.[51] Likewise, a new study suggested that cortisol regulates portal 5-HT levels.[173] Moreover, factors associated with altered gut permeability or worsening of the gut−brain axis structure (e.g., leaky gut or leaky BBB) may converge as a systemic process, in which many compounds spanning disparate chemical pools are likely to be involved.[36,112]

4.3. Opportunities and Challenges. With recent data collectively supporting an active microbiota−brain axis, targeting gut microbiota and the neuroactive metabolites naturally arises as an appealing approach for achieving improved neurophysiological states, cognitive functions, emotional states, and behaviors.[174−176] In this light, frameworks for drug development are transformed to incorporate NGS-driven data mining efforts of the microbiome for novel discovery, compound screening, and the design of synthetic biology strategies, with the goal of counteracting neurological disorders[32,139] or bacterially mediated side effects of drug treatment.[68,177] In parallel, the nutrition and food industries have started leveraging the effects and use of gut microbiota to design novel and healthier diets,[178] with applications ranging from development of neuroprotective prebiotics and probiotics (together coined psychobiotics) for achieving improved psychological states even in healthy normal individuals[19] to the design of a ketogenic diet for antiseizure benefits;[179] in parallel, a dynamic mouse peptidome landscape was constructed in response to probiotic modulation of the gut−brain axis and could provide value for future probiotics research toward better mental health.[180] In the public health arena, environmental toxicological research may target gut microbiota as one functional entity (or "organ") in mediating the fate and neurotoxic effects of ever-prevailing environmental toxicants, including pesticides,[181] bisphenol A,[182] and nanoparticles.[183]

Challenges will be encountered in advancing the microbiota−gut−brain axis field. To date, the molecular underpinnings of such crosstalk remain largely unelucidated and are hindered by methodological limitations in the standardization of neuro-phenotyping,[24] development of relevant *in vivo* models,[184] microbiota characterization,[185] microbial source apportionment (for example, from host or direct food intake),[186] confounder adjustment,[25] neurochemistry analysis,[187] etc. Given the bidirectional, dynamic, and elastic nature of the gut microbiota, it is inherently challenging to define their neuroactive potential in quantitative proxies. To resolve this, the 56 gut−brain biochemical modules curated in the recent FGFP cohort study[25] may be a valuable reference, with each module referring to a biochemical step (for production or degradation) of a singular neurochemical. Beyond that, sequencing-based approaches, on which most existing microbiome studies heavily relied, encounter both experimental and computational challenges to achieve accurate and definitive characterization of the gut microbiome;[188] best practices, protocols, and standards were proposed.[188,189]

Importantly, given that current insights are mostly derived from animal studies, result extrapolation from animals to the human population can be problematic. Specifically, fundamental gaps remain in terms of whether mice are good models for human microbiome alongside other emerging animal models[190] and to what extent the mouse-to-human inference can be made that establishes in humans valid and meaningful cause−effect relationships of the microbiota without overextrapolation, which is surprisingly common (95%; 36 of 38) as assessed by a recent systematic review article.[191] Through systematic analysis of existing translational studies using human microbiota-associated rodent models, Walters and colleagues identified most critical aspects for advancing interspecies inference as (i) insufficient rigor in experimental design and statistics and (ii) a paucity of negative results in the scientific community where oftentimes only positive findings are published.[191] New machine learning models have thus been proposed for advancing mouse-to-human inference,[192,193] and more large-scale human epidemiological studies are needed to test and validate findings based on mouse models, especially those coupled with high-throughput omics screening approaches (e.g., metabolomics, metaproteomics, and metagenomics).[187,194] Even for compounds with well-validated neuroactive effects (e.g., SCFAs), caution needs to be taken concerning dose levels and routes of delivery when testing in both experimental settings and clinical trials to ensure safety and efficacy. This entails knowledge of pharmacokinetic/toxicokinetic modeling, the critical time window for dosage, individual disease susceptibility and comorbidity, intra- and interindividual variability of gut microbial dynamics, etc. In a nutshell, to advance the microbiota and gut−brain axis field, future work needs to be undertaken to examine (i) our fundamental understanding and definition of the gut microbiome, gut−brain axis, human health, and related neurologic/mental diseases,[195] (ii) developing comprehensive, unbiased, and definitive approaches in characterizing the gut microbiome and health proxies,[188] (iii) enhancing rigor in experimental design and statistics while promoting reporting of negative results for valid and unbiased animal-to-human inference,[191] and (iv) multiomics integration across multiple cohorts and experimental models for biomarker discovery and validation.[196]

5. CONCLUDING REMARKS

Recent studies collectively support an active functional role of the gut microbiota in modulating human neurophysiological states, emotions, and behaviors, opening doors of targeting microbiota and their neuroactive metabolites for improved

neurologic/mental health. In this Perspective, we focus on the biochemical aspects of the microbiota−brain axis by reviewing microbial molecules spanning small-molecule and larger neuropeptides. We summarized for these molecules the biosynthesis, uptake, activities, and functions as mediated by the gut microbiota, turned to disease models and cohort studies to carefully evaluate their neuroactive roles, and discussed opportunities and challenges toward mechanistic elucidation and therapeutic application. Future studies revolving around finding novel microbial molecules of gut−brain signaling may entail multiomics mining efforts in parallel with hypothesis-driven research on interclass compound interaction.

AUTHOR INFORMATION

Corresponding Author

Kun Lu − *Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States; Curriculum in Toxicology and Environmental Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States;* orcid.org/0000-0002-8125-2394; Email: kunlu@unc.edu

Authors

Yunjia Lai − *Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States;* orcid.org/0000-0002-1081-0897

Radhika Dhingra − *Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States; Institute of Environmental Health Solutions, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States*

Zhenfa Zhang − *Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States*

Louise M. Ball − *Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States*

Mark J. Zylka − *UNC Neuroscience Center and Department of Cell and Molecular Physiology, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States; Carolina Institute for Developmental Disabilities, The University of North Carolina at Chapel Hill, Carrboro, North Carolina 27510, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.biochem.1c00656

Funding

This work was supported by the National Institute of Environmental Health Sciences (NIEHS) via Grants R01ES024950, R03ES032067, P30ES010126, and P42ES031007. M.J.Z. is supported by NIEHS Grant R35ES028366. Y.L. also acknowledges partial funding from the Chen-Yu Yen and Whay-Ray C. Yen Graduate Fellowship of UNC Gillings School of Global Public Health (2018−2019).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank all of the scientists who have inspired and contributed to this fledging field linking microbiota to the gut−brain axis.

REFERENCES

(1) Turnbaugh, P. J.; Ley, R. E.; Hamady, M.; Fraser-Liggett, C. M.; Knight, R.; Gordon, J. I. The human microbiome project. *Nature* **2007**, *449* (7164), 804−10.

(2) Turnbaugh, P. J.; Ley, R. E.; Mahowald, M. A.; Magrini, V.; Mardis, E. R.; Gordon, J. I. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **2006**, *444* (7122), 1027−1031.

(3) Ramakrishna, B. S. Role of the gut microbiota in human nutrition and metabolism. *J. Gastroenterol. Hepatol.* **2013**, *28* (Suppl. 4), 9−17.

(4) Hooper, L. V.; Littman, D. R.; Macpherson, A. J. Interactions Between the Microbiota and the Immune System. *Science* **2012**, *336* (6086), 1268−1273.

(5) Neuman, H.; Debelius, J. W.; Knight, R.; Koren, O. Microbial endocrinology: the interplay between the microbiota and the endocrine system. *FEMS Microbiology Reviews* **2015**, *39* (4), 509−521.

(6) Wells, J. M.; Rossi, O.; Meijerink, M.; van Baarlen, P. Epithelial crosstalk at the microbiota−mucosal interface. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (Suppl. 1), 4607−4614.

(7) Guo, H.; Chou, W.-C.; Lai, Y.; Liang, K.; Tam, J. W.; Brickey, W. J.; Chen, L.; Montgomery, N. D.; Li, X.; Bohannon, L. M.; Sung, A. D.; Chao, N. J.; Peled, J. U.; Gomes, A. L. C.; van den Brink, M. R. M.; French, M. J.; Macintyre, A. N.; Sempowski, G. D.; Tan, X.; Sartor, R. B.; Lu, K.; Ting, J. P. Y. Multi-omics analyses of radiation survivors identify radioprotective microbes and metabolites. *Science* **2020**, *370* (6516), eaay9097.

(8) Hsiao, E. Y.; McBride, S. W.; Hsien, S.; Sharon, G.; Hyde, E. R.; McCue, T.; Codelli, J. A.; Chow, J.; Reisman, S. E.; Petrosino, J. F.; Patterson, P. H.; Mazmanian, S. K. Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders. *Cell* **2013**, *155* (7), 1451−1463.

(9) Bermudez-Martin, P.; Becker, J. A. J.; Caramello, N.; Fernandez, S. P.; Costa-Campos, R.; Canaguier, J.; Barbosa, S.; Martinez-Gili, L.; Myridakis, A.; Dumas, M.-E.; Bruneau, A.; Cherbuy, C.; Langella, P.; Callebert, J.; Launay, J.-M.; Chabry, J.; Barik, J.; Le Merrer, J.; Glaichenhaus, N.; Davidovic, L. The microbial metabolite p-Cresol induces autistic-like behaviors in mice by remodeling the gut microbiota. *Microbiome* **2021**, *9* (1), 157.

(10) Peralta-Marzal, L. N.; Prince, N.; Bajic, D.; Roussin, L.; Naudon, L.; Rabot, S.; Garssen, J.; Kraneveld, A. D.; Perez-Pardo, P. The Impact of Gut Microbiota-Derived Metabolites in Autism Spectrum Disorders. *Int. J. Mol. Sci.* **2021**, *22* (18), 10052.

(11) Wang, H.; Yang, F.; Zhang, S.; Xin, R.; Sun, Y. Genetic and environmental factors in Alzheimer's and Parkinson's diseases and promising therapeutic intervention via fecal microbiota transplantation. *npj Parkinson's Dis.* **2021**, *7* (1), 70.

(12) Chen, C.; Ahn, E. H.; Kang, S. S.; Liu, X.; Alam, A.; Ye, K. Gut dysbiosis contributes to amyloid pathology, associated with C/EBPβ/AEP signaling activation in Alzheimer's disease mouse model. *Sci. Adv.* **2020**, *6* (31), eaba0466.

(13) Erny, D.; Dokalis, N.; Mezo, C.; Castoldi, A.; Mossad, O.; Staszewski, O.; Frosch, M.; Villa, M.; Fuchs, V.; Mayer, A.; Neuber, J.; Sosat, J.; Tholen, S.; Schilling, O.; Vlachos, A.; Blank, T.; Gomez de Agüero, M.; Macpherson, A. J.; Pearce, E. J.; Prinz, M. Microbiota-derived acetate enables the metabolic fitness of the brain innate

immune system during health and disease. *Cell Metab.* **2021**, *33* (11), 2260−2276.e7.
(14) Blacher, E.; Bashiardes, S.; Shapiro, H.; Rothschild, D.; Mor, U.; Dori-Bachash, M.; Kleimeyer, C.; Moresi, C.; Harnik, Y.; Zur, M.; Zabari, M.; Brik, R. B.-Z.; Kviatcovsky, D.; Zmora, N.; Cohen, Y.; Bar, N.; Levi, I.; Amar, N.; Mehlman, T.; Brandis, A.; Biton, I.; Kuperman, Y.; Tsoory, M.; Alfahel, L.; Harmelin, A.; Schwartz, M.; Israelson, A.; Arike, L.; Johansson, M. E. V.; Hansson, G. C.; Gotkine, M.; Segal, E.; Elinav, E. Potential roles of gut microbiome and metabolites in modulating ALS in mice. *Nature* **2019**, *572* (7770), 474−480.
(15) Ghezzi, L.; Cantoni, C.; Pinget, G. V.; Zhou, Y.; Piccio, L. Targeting the gut to treat multiple sclerosis. *J. Clin. Invest.* **2021**, *131* (13), n/a DOI: 10.1172/JCI143774.
(16) Foster, J. A.; McVey Neufeld, K.-A. Gut−brain axis: how the microbiome influences anxiety and depression. *Trends Neurosci.* **2013**, *36* (5), 305−312.
(17) Powell, N.; Walker, M. M.; Talley, N. J. The mucosal immune system: master regulator of bidirectional gut-brain communications. *Nat. Rev. Gastroenterol. Hepatol.* **2017**, *14* (3), 143−159.
(18) Fung, T. C.; Olson, C. A.; Hsiao, E. Y. Interactions between the microbiota, immune and nervous systems in health and disease. *Nat. Neurosci.* **2017**, *20* (2), 145−155.
(19) Dinan, T. G.; Cryan, J. F. The Microbiome-Gut-Brain Axis in Health and Disease. *Gastroenterol Clin North Am.* **2017**, *46* (1), 77−89.
(20) Sudo, N.; Chida, Y.; Aiba, Y.; Sonoda, J.; Oyama, N.; Yu, X. N.; Kubo, C.; Koga, Y. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J. Physiol.* **2004**, *558* (1), 263−275.
(21) Bravo, J. A.; Forsythe, P.; Chew, M. V.; Escaravage, E.; Savignac, H. M.; Dinan, T. G.; Bienenstock, J.; Cryan, J. F. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (38), 16050−5.
(22) Boehme, M.; Guzzetta, K. E.; Bastiaanssen, T. F. S.; van de Wouw, M.; Moloney, G. M.; Gual-Grau, A.; Spichak, S.; Olavarría-Ramírez, L.; Fitzgerald, P.; Morillas, E.; Ritz, N. L.; Jaggar, M.; Cowan, C. S. M.; Crispie, F.; Donoso, F.; Halitzki, E.; Neto, M. C.; Sichetti, M.; Golubeva, A. V.; Fitzgerald, R. S.; Claesson, M. J.; Cotter, P. D.; O'Leary, O. F.; Dinan, T. G.; Cryan, J. F. Microbiota from young mice counteracts selective age-associated behavioral deficits. *Nature Aging* **2021**, *1* (8), 666−676.
(23) Kundu, P.; Lee, H. U.; Garcia-Perez, I.; Tay, E. X. Y.; Kim, H.; Faylon, L. E.; Martin, K. A.; Purbojati, R.; Drautz-Moses, D. I.; Ghosh, S.; Nicholson, J. K.; Schuster, S.; Holmes, E.; Pettersson, S. Neurogenesis and prolongevity signaling in young germ-free mice transplanted with the gut microbiota of old mice. *Sci. Transl. Med.* **2019**, *11* (518), eaau4760.
(24) Hooks, K. B.; Konsman, J. P.; O'Malley, M. A. Microbiota-gut-brain research: a critical analysis. *Behav Brain Sci.* **2019**, *42*, 1−40.
(25) Valles-Colomer, M.; Falony, G.; Darzi, Y.; Tigchelaar, E. F.; Wang, J.; Tito, R. Y.; Schiweck, C.; Kurilshikov, A.; Joossens, M.; Wijmenga, C.; Claes, S.; Van Oudenhove, L.; Zhernakova, A.; Vieira-Silva, S.; Raes, J. The neuroactive potential of the human gut microbiota in quality of life and depression. *Nat. Microbiol* **2019**, *4* (4), 623−632.
(26) Zhu, F.; Ju, Y.; Wang, W.; Wang, Q.; Guo, R.; Ma, Q.; Sun, Q.; Fan, Y.; Xie, Y.; Yang, Z.; Jie, Z.; Zhao, B.; Xiao, L.; Yang, L.; Zhang, T.; Feng, J.; Guo, L.; He, X.; Chen, Y.; Chen, C.; Gao, C.; Xu, X.; Yang, H.; Wang, J.; Dang, Y.; Madsen, L.; Brix, S.; Kristiansen, K.; Jia, H.; Ma, X. Metagenome-wide association of gut microbiome features for schizophrenia. *Nat. Commun.* **2020**, *11* (1), 1612.
(27) Kimmel, M.; Jin, W.; Xia, K.; Lun, K.; Azcarate-Peril, A.; Plantinga, A.; Wu, M.; Ataei, S.; Rackers, H.; Carroll, I.; Meltzer-Brody, S.; Fransson, E.; Knickmeyer, R. Metabolite trajectories across the perinatal period and mental health: A preliminary study of tryptophan-related metabolites, bile acids and microbial composition. *Behav. Brain Res.* **2022**, *418*, 113635.
(28) Rogers, G. B.; Keating, D. J.; Young, R. L.; Wong, M. L.; Licinio, J.; Wesselingh, S. From gut dysbiosis to altered brain function and mental illness: mechanisms and pathways. *Mol. Psychiatry* **2016**, *21* (6), 738−48.
(29) Agirman, G.; Hsiao, E. Y. SnapShot: The microbiota-gut-brain axis. *Cell* **2021**, *184* (9), 2524−2524.e1.
(30) Lai, Y.; Liu, C. W.; Yang, Y.; Hsiao, Y. C.; Ru, H.; Lu, K. High-coverage metabolomics uncovers microbiota-driven biochemical landscape of interorgan transport and gut-brain communication in mice. *Nat. Commun.* **2021**, *12* (1), 6000.
(31) de la Fuente-Nunez, C.; Meneguetti, B. T.; Franco, O. L.; Lu, T. K. Neuromicrobiology: How Microbes Influence the Brain. *ACS Chem. Neurosci.* **2018**, *9* (2), 141−150.
(32) Donia, M. S.; Fischbach, M. A. Small molecules from the human microbiota. *Science* **2015**, *349* (6246), 1254766.
(33) Sharon, G.; Garg, N.; Debelius, J.; Knight, R.; Dorrestein, P. C.; Mazmanian, S. K. Specialized Metabolites from the Microbiome in Health and Disease. *Cell Metab.* **2014**, *20* (5), 719−730.
(34) Wikoff, W. R.; Anfora, A. T.; Liu, J.; Schultz, P. G.; Lesley, S. A.; Peters, E. C.; Siuzdak, G. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106* (10), 3698−3703.
(35) Nicholson, J. K.; Holmes, E.; Kinross, J.; Burcelin, R.; Gibson, G.; Jia, W.; Pettersson, S. Host-gut microbiota metabolic interactions. *Science* **2012**, *336* (6086), 1262−7.
(36) Kelly, J.; Kennedy, P.; Cryan, J.; Dinan, T.; Clarke, G.; Hyland, N. Breaking Down the Barriers: The Gut Microbiome, Intestinal Permeability and Stress-related Psychiatric Disorders. *Front. Cell. Neurosci.* **2015**, *9* (392), 392 DOI: 10.3389/fncel.2015.00392.
(37) Veiga-Fernandes, H.; Pachnis, V. Neuroimmune regulation during intestinal development and homeostasis. *Nat. Immunol.* **2017**, *18* (2), 116−122.
(38) Pokusaeva, K.; Johnson, C.; Luk, B.; Uribe, G.; Fu, Y.; Oezguen, N.; Matsunami, R. K.; Lugo, M.; Major, A.; Mori-Akiyama, Y.; Hollister, E. B.; Dann, S. M.; Shi, X. Z.; Engler, D. A.; Savidge, T.; Versalovic, J. GABA-producing Bifidobacterium dentium modulates visceral sensitivity in the intestine. *Neurogastroenterol. Motil.* **2017**, *29* (1), e12904.
(39) Wu, S.; Bekhit, A. E.-D. A.; Wu, Q.; Chen, M.; Liao, X.; Wang, J.; Ding, Y. Bioactive peptides and gut microbiota: Candidates for a novel strategy for reduction and control of neurodegenerative diseases. *Trends Food Sci. Technol.* **2021**, *108*, 164−176.
(40) Meng, D.; Sommella, E.; Salviati, E.; Campiglia, P.; Ganguli, K.; Djebali, K.; Zhu, W.; Walker, W. A. Indole-3-lactic acid, a metabolite of tryptophan, secreted by Bifidobacterium longum subspecies infantis is anti-inflammatory in the immature intestine. *Pediatr. Res.* **2020**, *88* (2), 209−217.
(41) den Besten, G.; van Eunen, K.; Groen, A. K.; Venema, K.; Reijngoud, D. J.; Bakker, B. M. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J. Lipid Res.* **2013**, *54* (9), 2325−40.
(42) Koh, A.; De Vadder, F.; Kovatcheva-Datchary, P.; Backhed, F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell* **2016**, *165* (6), 1332−1345.
(43) Morrison, D. J.; Preston, T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes* **2016**, *7* (3), 189−200.
(44) Flint, H. J.; Duncan, S. H.; Scott, K. P.; Louis, P. Links between diet, gut microbiota composition and gut metabolism. *Proc. Nutr. Soc.* **2015**, *74* (1), 13−22.
(45) Vital, M.; Howe, A. C.; Tiedje, J. M. Revealing the Bacterial Butyrate Synthesis Pathways by Analyzing (Meta)genomic Data. *mBio* **2014**, *5* (2), e00889−14.
(46) Bjursell, M.; Admyre, T.; Göransson, M.; Marley, A. E.; Smith, D. M.; Oscarsson, J.; Bohlooly-Y, M. Improved glucose control and reduced body fat mass in free fatty acid receptor 2-deficient mice fed a high-fat diet. *American Journal of Physiology-Endocrinology and Metabolism* **2011**, *300* (1), E211−E220.

(47) Kovatcheva-Datchary, P.; Nilsson, A.; Akrami, R.; Lee, Y. S.; De Vadder, F.; Arora, T.; Hallen, A.; Martens, E.; Björck, I.; Bäckhed, F. Dietary Fiber-Induced Improvement in Glucose Metabolism Is Associated with Increased Abundance of Prevotella. *Cell Metab.* **2015**, *22* (6), 971−982.
(48) Schwiertz, A.; Taras, D.; Schäfer, K.; Beijer, S.; Bos, N. A.; Donus, C.; Hardt, P. D. Microbiota and SCFA in Lean and Overweight Healthy Subjects. *Obesity* **2010**, *18* (1), 190−195.
(49) Ríos-Covián, D.; Ruas-Madiedo, P.; Margolles, A.; Gueimonde, M.; de los Reyes-Gavilán, C. G.; Salazar, N. Intestinal Short Chain Fatty Acids and their Link with Diet and Human Health. *Front. Microbiol.* **2016**, *7*, 185 DOI: 10.3389/fmicb.2016.00185.
(50) Smith, P. M.; Howitt, M. R.; Panikov, N.; Michaud, M.; Gallini, C. A.; Bohlooly-Y, M.; Glickman, J. N.; Garrett, W. S. The Microbial Metabolites, Short-Chain Fatty Acids, Regulate Colonic Treg Cell Homeostasis. *Science* **2013**, *341* (6145), 569−573.
(51) Reigstad, C. S.; Salmonson, C. E.; Rainey, J. F., III; Szurszewski, J. H.; Linden, D. R.; Sonnenburg, J. L.; Farrugia, G.; Kashyap, P. C. Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *FASEB J.* **2015**, *29* (4), 1395−1403.
(52) Erny, D.; Hrabe de Angelis, A. L.; Jaitin, D.; Wieghofer, P.; Staszewski, O.; David, E.; Keren-Shaul, H.; Mahlakoiv, T.; Jakobshagen, K.; Buch, T.; Schwierzeck, V.; Utermohlen, O.; Chun, E.; Garrett, W. S.; McCoy, K. D.; Diefenbach, A.; Staeheli, P.; Stecher, B.; Amit, I.; Prinz, M. Host microbiota constantly control maturation and function of microglia in the CNS. *Nat. Neurosci.* **2015**, *18* (7), 965−77.
(53) Braniste, V.; Al-Asmakh, M.; Kowal, C.; Anuar, F.; Abbaspour, A.; Tóth, M.; Korecka, A.; Bakocevic, N.; Ng, L. G.; Kundu, P.; Gulyás, B.; Halldin, C.; Hultenby, K.; Nilsson, H.; Hebert, H.; Volpe, B. T.; Diamond, B.; Pettersson, S. The gut microbiota influences blood-brain barrier permeability in mice. *Sci. Transl. Med.* **2014**, *6* (263), 263ra158−263ra158.
(54) Frost, G.; Sleeth, M. L.; Sahuri-Arisoylu, M.; Lizarbe, B.; Cerdan, S.; Brody, L.; Anastasovska, J.; Ghourab, S.; Hankir, M.; Zhang, S.; Carling, D.; Swann, J. R.; Gibson, G.; Viardot, A.; Morrison, D.; Louise Thomas, E.; Bell, J. D. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat. Commun.* **2014**, *5*, 3611.
(55) Sampson, T. R.; Debelius, J. W.; Thron, T.; Janssen, S.; Shastri, G. G.; Ilhan, Z. E.; Challis, C.; Schretter, C. E.; Rocha, S.; Gradinaru, V.; Chesselet, M. F.; Keshavarzian, A.; Shannon, K. M.; Krajmalnik-Brown, R.; Wittung-Stafshede, P.; Knight, R.; Mazmanian, S. K. Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. *Cell* **2016**, *167* (6), 1469−1480.e12.
(56) Perry, R. J.; Peng, L.; Barry, N. A.; Cline, G. W.; Zhang, D.; Cardone, R. L.; Petersen, K. F.; Kibbey, R. G.; Goodman, A. L.; Shulman, G. I. Acetate mediates a microbiome-brain-beta-cell axis to promote metabolic syndrome. *Nature* **2016**, *534* (7606), 213−7.
(57) Al-Asmakh, M.; Hedin, L. Microbiota and the control of blood-tissue barriers. *Tissue Barriers* **2015**, *3* (3), e1039691.
(58) Nøhr, M. K.; Pedersen, M. H.; Gille, A.; Egerod, K. L.; Engelstoft, M. S.; Husted, A. S.; Sichlau, R. M.; Grunddal, K. V.; Seier Poulsen, S.; Han, S.; Jones, R. M.; Offermanns, S.; Schwartz, T. W. GPR41/FFAR3 and GPR43/FFAR2 as Cosensors for Short-Chain Fatty Acids in Enteroendocrine Cells vs FFAR3 in Enteric Neurons and FFAR2 in Enteric Leukocytes. *Endocrinology* **2013**, *154* (10), 3552−3564.
(59) Hofmann, A. F. The Continuing Importance of Bile Acids in Liver and Intestinal Disease. *Arch. Intern. Med.* **1999**, *159* (22), 2647−2658.
(60) Quinn, R. A.; Melnik, A. V.; Vrbanac, A.; Fu, T.; Patras, K. A.; Christy, M. P.; Bodai, Z.; Belda-Ferre, P.; Tripathi, A.; Chung, L. K.; Downes, M.; Welch, R. D.; Quinn, M.; Humphrey, G.; Panitchpakdi, M.; Weldon, K. C.; Aksenov, A.; da Silva, R.; Avila-Pacheco, J.; Clish, C.; Bae, S.; Mallick, H.; Franzosa, E. A.; Lloyd-Price, J.; Bussell, R.; Thron, T.; Nelson, A. T.; Wang, M.; Leszczynski, E.; Vargas, F.; Gauglitz, J. M.; Meehan, M. J.; Gentry, E.; Arthur, T. D.; Komor, A. C.; Poulsen, O.; Boland, B. S.; Chang, J. T.; Sandborn, W. J.; Lim, M.; Garg, N.; Lumeng, J. C.; Xavier, R. J.; Kazmierczak, B. I.; Jain, R.; Egan, M.; Rhee, K. E.; Ferguson, D.; Raffatellu, M.; Vlamakis, H.; Haddad, G. G.; Siegel, D.; Huttenhower, C.; Mazmanian, S. K.; Evans, R. M.; Nizet, V.; Knight, R.; Dorrestein, P. C. Global chemical effects of the microbiome include new bile-acid conjugations. *Nature* **2020**, *579* (7797), 123−129.
(61) Kuipers, F.; Bloks, V. W.; Groen, A. K. Beyond intestinal soap–bile acids in metabolic control. *Nat. Rev. Endocrinol.* **2014**, *10* (8), 488−98.
(62) Sun, J.; Chang, E. B. Exploring gut microbes in human health and disease: Pushing the envelope. *Genes Dis* **2014**, *1* (2), 132−139.
(63) Wahlstrom, A.; Sayin, S. I.; Marschall, H. U.; Backhed, F. Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism. *Cell Metab.* **2016**, *24* (1), 41−50.
(64) Lefebvre, P.; Cariou, B.; Lien, F.; Kuipers, F.; Staels, B. Role of Bile Acids and Bile Acid Receptors in Metabolic Regulation. *Physiol. Rev.* **2009**, *89* (1), 147−191.
(65) Arab, J. P.; Karpen, S. J.; Dawson, P. A.; Arrese, M.; Trauner, M. Bile acids and nonalcoholic fatty liver disease: Molecular insights and therapeutic perspectives. *Hepatology* **2017**, *65* (1), 350−362.
(66) Schroeder, B. O.; Backhed, F. Signals from the gut microbiota to distant organs in physiology and disease. *Nat. Med.* **2016**, *22* (10), 1079−1089.
(67) Bernstein, H.; Bernstein, C.; Payne, C. M.; Dvorakova, K.; Garewal, H. Bile acids as carcinogens in human gastrointestinal cancers. *Mutat. Res., Rev. Mutat. Res.* **2005**, *589* (1), 47−65.
(68) Bhatt, A. P.; Redinbo, M. R.; Bultman, S. J. The role of the microbiome in cancer development and therapy. *Ca-Cancer J. Clin.* **2017**, *67* (4), 326−344.
(69) Guzior, D. V.; Quinn, R. A. Review: microbial transformations of human bile acids. *Microbiome* **2021**, *9* (1), 140.
(70) Swann, J. R.; Want, E. J.; Geier, F. M.; Spagou, K.; Wilson, I. D.; Sidaway, J. E.; Nicholson, J. K.; Holmes, E. Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (Suppl. 1), 4523−4530.
(71) Sayin, S. I.; Wahlstrom, A.; Felin, J.; Jantti, S.; Marschall, H. U.; Bamberg, K.; Angelin, B.; Hyotylainen, T.; Oresic, M.; Backhed, F. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab.* **2013**, *17* (2), 225−35.
(72) Liu, S.; Marcelin, G.; Blouet, C.; Jeong, J. H.; Jo, Y. H.; Schwartz, G. J.; Chua, S., Jr. A gut-brain axis regulating glucose metabolism mediated by bile acids and competitive fibroblast growth factor actions at the hypothalamus. *Mol. Metab.* **2018**, *8*, 37−50.
(73) Parséus, A.; Sommer, N.; Sommer, F.; Caesar, R.; Molinaro, A.; Ståhlman, M.; Greiner, T. U.; Perkins, R.; Bäckhed, F. Microbiota-induced obesity requires farnesoid X receptor. *Gut* **2017**, *66* (3), 429−437.
(74) Li, J. V.; Ashrafian, H.; Bueter, M.; Kinross, J.; Sands, C.; le Roux, C. W.; Bloom, S. R.; Darzi, A.; Athanasiou, T.; Marchesi, J. R.; Nicholson, J. K.; Holmes, E. Metabolic surgery profoundly influences gut microbial-host metabolic cross-talk. *Gut* **2011**, *60* (9), 1214−23.
(75) Dixon, J. B.; Lambert, E. A.; Lambert, G. W. Neuroendocrine adaptations to bariatric surgery. *Mol. Cell. Endocrinol.* **2015**, *418* (2), 143−152.
(76) Bajaj, J. S. Alcohol, liver disease and the gut microbiota. *Nat. Rev. Gastroenterol. Hepatol.* **2019**, *16* (4), 235−246.
(77) Golubeva, A. V.; Joyce, S. A.; Moloney, G.; Burokas, A.; Sherwin, E.; Arboleya, S.; Flynn, I.; Khochanskiy, D.; Moya-Perez, A.; Peterson, V.; Rea, K.; Murphy, K.; Makarova, O.; Buravkov, S.; Hyland, N. P.; Stanton, C.; Clarke, G.; Gahan, C. G. M.; Dinan, T. G.; Cryan, J. F. Microbiota-related Changes in Bile Acid & Tryptophan Metabolism are Associated with Gastrointestinal Dysfunction in a Mouse Model of Autism. *EBioMedicine* **2017**, *24*, 166−178.
(78) Reddy, I. A.; Smith, N. K.; Erreger, K.; Ghose, D.; Saunders, C.; Foster, D. J.; Turner, B.; Poe, A.; Albaugh, V. L.; McGuinness, O.; Hackett, T. A.; Grueter, B. A.; Abumrad, N. N.; Flynn, C. R.; Galli, A.

Bile diversion, a bariatric surgery, and bile acid signaling reduce central cocaine reward. *PLoS Biol.* **2018**, *16* (7), e2006682.
(79) Perino, A.; Velazquez-Villegas, L. A.; Bresciani, N.; Sun, Y.; Huang, Q.; Fenelon, V. S.; Castellanos-Jankiewicz, A.; Zizzari, P.; Bruschetta, G.; Jin, S.; Baleisyte, A.; Gioiello, A.; Pellicciari, R.; Ivanisevic, J.; Schneider, B. L.; Diano, S.; Cota, D.; Schoonjans, K. Central anorexigenic actions of bile acids are mediated by TGR5. *Nat. Metab* **2021**, *3* (5), 595−603.
(80) MahmoudianDehkordi, S.; Arnold, M.; Nho, K.; Ahmad, S.; Jia, W.; Xie, G.; Louie, G.; Kueider-Paisley, A.; Moseley, M. A.; Thompson, J. W.; St John Williams, L.; Tenenbaum, J. D.; Blach, C.; Baillie, R.; Han, X.; Bhattacharyya, S.; Toledo, J. B.; Schafferer, S.; Klein, S.; Koal, T.; Risacher, S. L.; Kling, M. A.; Motsinger-Reif, A.; Rotroff, D. M.; Jack, J.; Hankemeier, T.; Bennett, D. A.; De Jager, P. L.; Trojanowski, J. Q.; Shaw, L. M.; Weiner, M. W.; Doraiswamy, P. M.; van Duijn, C. M.; Saykin, A. J.; Kastenmuller, G.; Kaddurah-Daouk, R. Altered bile acid profile associates with cognitive impairment in Alzheimer's disease-An emerging role for gut microbiome. *Alzheimer's Dementia* **2019**, *15* (1), 76−92.
(81) Nho, K.; Kueider-Paisley, A.; MahmoudianDehkordi, S.; Arnold, M.; Risacher, S. L.; Louie, G.; Blach, C.; Baillie, R.; Han, X.; Kastenmuller, G.; Jia, W.; Xie, G.; Ahmad, S.; Hankemeier, T.; van Duijn, C. M.; Trojanowski, J. Q.; Shaw, L. M.; Weiner, M. W.; Doraiswamy, P. M.; Saykin, A. J.; Kaddurah-Daouk, R. Altered bile acid profile in mild cognitive impairment and Alzheimer's disease: Relationship to neuroimaging and CSF biomarkers. *Alzheimer's Dementia* **2019**, *15* (2), 232−244.
(82) Kamp, F.; Hamilton, J. A. Movement of fatty acids, fatty acid analogs, and bile acids across phospholipid bilayers. *Biochemistry* **1993**, *32* (41), 11074−11085.
(83) Higashi, T.; Watanabe, S.; Tomaru, K.; Yamazaki, W.; Yoshizawa, K.; Ogawa, S.; Nagao, H.; Minato, K.; Maekawa, M.; Mano, N. Unconjugated bile acids in rat brain: Analytical method based on LC/ESI-MS/MS with chemical derivatization and estimation of their origin by comparison to serum levels. *Steroids* **2017**, *125*, 107−113.
(84) Parry, G. J.; Rodrigues, C. M.; Aranha, M. M.; Hilbert, S. J.; Davey, C.; Kelkar, P.; Low, W. C.; Steer, C. J. Safety, tolerability, and cerebrospinal fluid penetration of ursodeoxycholic Acid in patients with amyotrophic lateral sclerosis. *Clin. Neuropharmacol.* **2010**, *33* (1), 17−21.
(85) Mertens, K. L.; Kalsbeek, A.; Soeters, M. R.; Eggink, H. M. Bile Acid Signaling Pathways from the Enterohepatic Circulation to the Central Nervous System. *Front. Neurosci.* **2017**, *11*, 617.
(86) Quinn, M.; DeMorrow, S. Bile in the Brain? A Role for Bile Acids in the Central Nervous System. *J. Cell Sci. Ther.* **2012**, *3*, 7 DOI: 10.4172/2157-7013.1000e113.
(87) Wu, J.; Zhu, X.; Lin, H.; Chen, Z.; Tang, H.; Wang, Y. Gender differences in the bile acid profiles of APP/PS1 transgenic AD mice. *Brain Res. Bull.* **2020**, *161*, 116−126.
(88) Cervenka, I.; Agudelo, L. Z.; Ruas, J. L. Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health. *Science* **2017**, *357* (6349), eaaf9794 DOI: 10.1126/science.aaf9794.
(89) Ruddick, J. P.; Evans, A. K.; Nutt, D. J.; Lightman, S. L.; Rook, G. A. W.; Lowry, C. A. Tryptophan metabolism in the central nervous system: medical implications. *Expert Rev. Mol. Med.* **2006**, *8* (20), 1−27.
(90) McMenamy, R. H.; Oncley, J. L. The Specific Binding of l-Tryptophan to Serum Albumin. *J. Biol. Chem.* **1958**, *233* (6), 1436−1447.
(91) Bender, D. A. Effects of a dietary excess of leucine on the metabolism of tryptophan in the rat: a mechanism for the pellagragenic action of leucine. *Br. J. Nutr.* **1983**, *50* (1), 25−32.
(92) Platten, M.; Nollen, E. A. A.; Rohrig, U. F.; Fallarino, F.; Opitz, C. A. Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond. *Nat. Rev. Drug Discovery* **2019**, *18* (5), 379−401.
(93) Clarke, G.; Grenham, S.; Scully, P.; Fitzgerald, P.; Moloney, R. D.; Shanahan, F.; Dinan, T. G.; Cryan, J. F. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol. Psychiatry* **2013**, *18* (6), 666−73.
(94) Li, Y.; Hao, Y.; Fan, F.; Zhang, B. The Role of Microbiome in Insomnia, Circadian Disturbance and Depression. *Frontiers in Psychiatry* **2018**, *9*, 669 DOI: 10.3389/fpsyt.2018.00669.
(95) Hubbard, T. D.; Murray, I. A.; Perdew, G. H. Indole and tryptophan metabolism: endogenous and dietary routes to Ah receptor activation. *Drug Metab. Dispos.* **2015**, *43*, 1522−1535.
(96) Arora, P. K.; Sharma, A.; Bae, H. Microbial Degradation of Indole and Its Derivatives. *J. Chem.* **2015**, *2015*, 1−13.
(97) Roager, H. M.; Licht, T. R. Microbial tryptophan catabolites in health and disease. *Nat. Commun.* **2018**, *9* (1), 3294.
(98) Agus, A.; Planchais, J.; Sokol, H. Gut Microbiota Regulation of Tryptophan Metabolism in Health and Disease. *Cell Host Microbe* **2018**, *23* (6), 716−724.
(99) Gershon, M. D.; Tack, J. The Serotonin Signaling System: From Basic Understanding To Drug Development for Functional GI Disorders. *Gastroenterology* **2007**, *132* (1), 397−414.
(100) Mercado, C. P.; Quintero, M. V.; Li, Y.; Singh, P.; Byrd, A. K.; Talabnin, K.; Ishihara, M.; Azadi, P.; Rusch, N. J.; Kuberan, B.; Maroteaux, L.; Kilic, F. A serotonin-induced N-glycan switch regulates platelet aggregation. *Sci. Rep.* **2013**, *3*, 2795.
(101) Yano, J. M.; Yu, K.; Donaldson, G. P.; Shastri, G. G.; Ann, P.; Ma, L.; Nagler, C. R.; Ismagilov, R. F.; Mazmanian, S. K.; Hsiao, E. Y. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* **2015**, *161* (2), 264−76.
(102) De Vadder, F.; Grasset, E.; Manneras Holm, L.; Karsenty, G.; Macpherson, A. J.; Olofsson, L. E.; Backhed, F. Gut microbiota regulates maturation of the adult enteric nervous system via enteric serotonin networks. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115* (25), 6458−6463.
(103) Kanai, M.; Funakoshi, H.; Takahashi, H.; Hayakawa, T.; Mizuno, S.; Matsumoto, K.; Nakamura, T. Tryptophan 2,3-dioxygenase is a key modulator of physiological neurogenesis and anxiety-related behavior in mice. *Mol. Brain* **2009**, *2* (1), 8.
(104) Guillemin, G. J.; Smythe, G.; Takikawa, O.; Brew, B. J. Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes, and neurons. *Glia* **2005**, *49* (1), 15−23.
(105) Müller, N.; Schwarz, M. J. The immune-mediated alteration of serotonin and glutamate: towards an integrated view of depression. *Mol. Psychiatry* **2007**, *12*, 988.
(106) Amori, L.; Guidetti, P.; Pellicciari, R.; Kajii, Y.; Schwarcz, R. On the relationship between the two branches of the kynurenine pathway in the rat brain in vivo. *J. Neurochem.* **2009**, *109* (2), 316−325.
(107) Albuquerque, E. X.; Schwarcz, R. Kynurenic acid as an antagonist of α7 nicotinic acetylcholine receptors in the brain: Facts and challenges. *Biochem. Pharmacol.* **2013**, *85* (8), 1027−1032.
(108) DiNatale, B. C.; Murray, I. A.; Schroeder, J. C.; Flaveny, C. A.; Lahoti, T. S.; Laurenzana, E. M.; Omiecinski, C. J.; Perdew, G. H. Kynurenic Acid Is a Potent Endogenous Aryl Hydrocarbon Receptor Ligand that Synergistically Induces Interleukin-6 in the Presence of Inflammatory Signaling. *Toxicol. Sci.* **2010**, *115* (1), 89−97.
(109) O'Mahony, S. M.; Clarke, G.; Borre, Y. E.; Dinan, T. G.; Cryan, J. F. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behav. Brain Res.* **2015**, *277*, 32−48.
(110) Chen, Y.; Guillemin, G. J. Kynurenine Pathway Metabolites in Humans: Disease and Healthy States. *Int. J. Tryptophan Res.* **2009**, *2*, IJTR.S2097.
(111) Gao, J.; Xu, K.; Liu, H.; Liu, G.; Bai, M.; Peng, C.; Li, T.; Yin, Y. Impact of the Gut Microbiota on Intestinal Immunity Mediated by Tryptophan Metabolism. *Front. Cell. Infect. Microbiol.* **2018**, *8*, 13.
(112) Venkatesh, M.; Mukherjee, S.; Wang, H.; Li, H.; Sun, K.; Benechet, A. P.; Qiu, Z.; Maher, L.; Redinbo, M. R.; Phillips, R. S.; Fleet, J. C.; Kortagere, S.; Mukherjee, P.; Fasano, A.; Le Ven, J.;

Nicholson, J. K.; Dumas, M. E.; Khanna, K. M.; Mani, S. Symbiotic Bacterial Metabolites Regulate Gastrointestinal Barrier Function via the Xenobiotic Sensor PXR and Toll-like Receptor 4. *Immunity* **2014**, *41* (2), 296−310.
(113) Wlodarska, M.; Luo, C.; Kolde, R.; d'Hennezel, E.; Annand, J. W.; Heim, C. E.; Krastel, P.; Schmitt, E. K.; Omar, A. S.; Creasey, E. A.; Garner, A. L.; Mohammadi, S.; O'Connell, D. J.; Abubucker, S.; Arthur, T. D.; Franzosa, E. A.; Huttenhower, C.; Murphy, L. O.; Haiser, H. J.; Vlamakis, H.; Porter, J. A.; Xavier, R. J. Indoleacrylic Acid Produced by Commensal Peptostreptococcus Species Suppresses Inflammation. *Cell Host Microbe* **2017**, *22* (1), 25−37.e6.
(114) Rothhammer, V.; Mascanfroni, I. D.; Bunse, L.; Takenaka, M. C.; Kenison, J. E.; Mayo, L.; Chao, C. C.; Patel, B.; Yan, R.; Blain, M.; Alvarez, J. I.; Kebir, H.; Anandasabapathy, N.; Izquierdo, G.; Jung, S.; Obholzer, N.; Pochet, N.; Clish, C. B.; Prinz, M.; Prat, A.; Antel, J.; Quintana, F. J. Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. *Nat. Med.* **2016**, *22* (6), 586−97.
(115) Osadchiy, V.; Labus, J. S.; Gupta, A.; Jacobs, J.; Ashe-McNalley, C.; Hsiao, E. Y.; Mayer, E. A. Correlation of tryptophan metabolites with connectivity of extended central reward network in healthy subjects. *PLoS One* **2018**, *13* (8), e0201772.
(116) Purves, D. *Neuroscience*; Sinauer Associates: Sunderland, MA, 2012.
(117) Strandwitz, P. Neurotransmitter modulation by the gut microbiota. *Brain Res.* **2018**, *1693*, 128−133.
(118) Mittal, R.; Debs, L. H.; Patel, A. P.; Nguyen, D.; Patel, K.; O'Connor, G.; Grati, M.; Mittal, J.; Yan, D.; Eshraghi, A. A.; Deo, S. K.; Daunert, S.; Liu, X. Z. Neurotransmitters: The Critical Modulators Regulating Gut-Brain Axis. *J. Cell. Physiol.* **2017**, *232* (9), 2359−2372.
(119) Lyte, M. Probiotics function mechanistically as delivery vehicles for neuroactive compounds: Microbial endocrinology in the design and use of probiotics. *BioEssays* **2011**, *33* (8), 574−81.
(120) Villageliu, D. N.; Rasmussen, S.; Lyte, M. A microbial endocrinology-based simulated small intestinal medium for the evaluation of neurochemical production by gut microbiota. *FEMS Microbiol. Ecol.* **2018**, *94* (7), n/a DOI: 10.1093/femsec/fiy096.
(121) Lyte, M. Microbial endocrinology and infectious disease in the 21st century. *Trends Microbiol.* **2004**, *12* (1), 14−20.
(122) Hertz, L. The Glutamate-Glutamine (GABA) Cycle: Importance of Late Postnatal Development and Potential Reciprocal Interactions between Biosynthesis and Degradation. *Front. Endocrinol. (Lausanne, Switz.)* **2013**, *4*, 59.
(123) Mazzoli, R.; Pessione, E. The Neuro-endocrinological Role of Microbial Glutamate and GABA Signaling. *Front. Microbiol.* **2016**, *7*, 1934.
(124) Janeczko, M. J.; Stoll, B.; Chang, X.; Guan, X.; Burrin, D. G. Extensive Gut Metabolism Limits the Intestinal Absorption of Excessive Supplemental Dietary Glutamate Loads in Infant Pigs. *J. Nutr.* **2007**, *137* (11), 2384−2390.
(125) Hawkins, R. A. The blood-brain barrier and glutamate. *Am. J. Clin. Nutr.* **2009**, *90* (3), 867S−874S.
(126) Cherlyn, S. Y. T.; Woon, P. S.; Liu, J. J.; Ong, W. Y.; Tsai, G. C.; Sim, K. Genetic association studies of glutamate, GABA and related genes in schizophrenia and bipolar disorder: A decade of advance. *Neurosci. Biobehav. Rev.* **2010**, *34* (6), 958−977.
(127) Boonstra, E.; de Kleijn, R.; Colzato, L. S.; Alkemade, A.; Forstmann, B. U.; Nieuwenhuis, S. Neurotransmitters as food supplements: the effects of GABA on brain and behavior. *Frontiers in Psychology* **2015**, *6*, 1520 DOI: 10.3389/fpsyg.2015.01520.
(128) Kakee, A.; Takanaga, H.; Terasaki, T.; Naito, M.; Tsuruo, T.; Sugiyama, Y. Efflux of a suppressive neurotransmitter, GABA, across the blood−brain barrier. *J. Neurochem.* **2001**, *79* (1), 110−118.
(129) Zheng, P.; Zeng, B.; Liu, M.; Chen, J.; Pan, J.; Han, Y.; Liu, Y.; Cheng, K.; Zhou, C.; Wang, H.; Zhou, X.; Gui, S.; Perry, S. W.; Wong, M.-L.; Licinio, J.; Wei, H.; Xie, P. The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice. *Science Advances* **2019**, *5* (2), eaau8317.
(130) Volkow, N. D.; Wise, R. A.; Baler, R. The dopamine motive system: implications for drug and food addiction. *Nat. Rev. Neurosci.* **2017**, *18* (12), 741−752.
(131) Fetissov, S. O. Role of the gut microbiota in host appetite control: bacterial growth to animal feeding behaviour. *Nat. Rev. Endocrinol.* **2017**, *13* (1), 11−25.
(132) Roshchina, V. V. Evolutionary Considerations of Neurotransmitters in Microbial, Plant, and Animal Cells. *Microbial Endocrinology* **2010**, 17−52.
(133) Liu, W. H.; Chuang, H. L.; Huang, Y. T.; Wu, C. C.; Chou, G. T.; Wang, S.; Tsai, Y. C. Alteration of behavior and monoamine levels attributable to Lactobacillus plantarum PS128 in germ-free mice. *Behav. Brain Res.* **2016**, *298*, 202−209.
(134) Diaz Heijtz, R.; Wang, S.; Anuar, F.; Qian, Y.; Bjorkholm, B.; Samuelsson, A.; Hibberd, M. L.; Forssberg, H.; Pettersson, S. Normal gut microbiota modulates brain development and behavior. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (7), 3047−3052.
(135) Kiraly, D. D.; Walker, D. M.; Calipari, E. S.; Labonte, B.; Issler, O.; Pena, C. J.; Ribeiro, E. A.; Russo, S. J.; Nestler, E. J. Alterations of the Host Microbiome Affect Behavioral Responses to Cocaine. *Sci. Rep.* **2016**, *6*, 35455.
(136) Moya-Perez, A.; Perez-Villalba, A.; Benítez-Páez, A.; Campillo, I.; Sanz, Y. Bifidobacterium CECT 7765 modulates early stress-induced immune, neuroendocrine and behavioral alterations in mice. *Brain, Behav., Immun.* **2017**, *65*, 43−56.
(137) Bedarf, J. R.; Hildebrand, F.; Coelho, L. P.; Sunagawa, S.; Bahram, M.; Goeser, F.; Bork, P.; Wullner, U. Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naive Parkinson's disease patients. *Genome Med.* **2017**, *9* (1), 39.
(138) Thomas, C. M.; Hong, T.; van Pijkeren, J. P.; Hemarajata, P.; Trinh, D. V.; Hu, W.; Britton, R. A.; Kalkum, M.; Versalovic, J. Histamine derived from probiotic Lactobacillus reuteri suppresses TNF via modulation of PKA and ERK signaling. *PLoS One* **2012**, *7* (2), e31951.
(139) Williams, B. B.; Van Benschoten, A. H.; Cimermancic, P.; Donia, M. S.; Zimmermann, M.; Taketani, M.; Ishihara, A.; Kashyap, P. C.; Fraser, J. S.; Fischbach, M. A. Discovery and characterization of gut microbiota decarboxylases that can produce the neurotransmitter tryptamine. *Cell Host Microbe* **2014**, *16* (4), 495−503.
(140) Pessione, E.; Pessione, A.; Lamberti, C.; Coïsson, D. J.; Riedel, K.; Mazzoli, R.; Bonetta, S.; Eberl, L.; Giunta, C. First evidence of a membrane-bound, tyramine and β-phenylethylamine producing, tyrosine decarboxylase in Enterococcus faecalis: A two-dimensional electrophoresis proteomic study. *Proteomics* **2009**, *9* (10), 2695−2710.
(141) Hulme, H.; Meikle, L. M.; Strittmatter, N.; van der Hooft, J. J. J.; Swales, J.; Bragg, R. A.; Villar, V. H.; Ormsby, M. J.; Barnes, S.; Brown, S. L.; Dexter, A.; Kamat, M. T.; Komen, J. C.; Walker, D.; Milling, S.; Osterweil, E. K.; MacDonald, A. S.; Schofield, C. J.; Tardito, S.; Bunch, J.; Douce, G.; Edgar, J. M.; Edrada-Ebel, R.; Goodwin, R. J. A.; Burchmore, R.; Wall, D. M. Microbiome-derived carnitine mimics as previously unknown mediators of gut-brain axis communication. *Sci. Adv.* **2020**, *6* (11), eaax6328.
(142) Motta, J.-P.; Wallace, J. L.; Buret, A. G.; Deraison, C.; Vergnolle, N. Gastrointestinal biofilms in health and disease. *Nat. Rev. Gastroenterol. Hepatol.* **2021**, *18* (5), 314−334.
(143) Goyal, A.; Wang, T.; Dubinkina, V.; Maslov, S. Ecology-guided prediction of cross-feeding interactions in the human gut microbiome. *Nat. Commun.* **2021**, *12* (1), 1335.
(144) Negi, S.; Das, D. K.; Pahari, S.; Nadeem, S.; Agrewala, J. N. Potential Role of Gut Microbiota in Induction and Regulation of Innate Immune Memory. *Front. Immunol.* **2019**, *10*, 2441−2441.
(145) Mu, C.; Yang, Y.; Zhu, W. Gut Microbiota: The Brain Peacekeeper. *Front. Microbiol.* **2016**, *7*, 345−345.

(146) Yoo, B. B.; Mazmanian, S. K. The Enteric Network: Interactions between the Immune and Nervous Systems of the Gut. *Immunity* **2017**, *46* (6), 910−926.
(147) Wu, M. L.; Yang, X. Q.; Xue, L.; Duan, W.; Du, J. R. Age-related cognitive decline is associated with microbiota-gut-brain axis disorders and neuroinflammation in mice. *Behav. Brain Res.* **2021**, *402*, 113125.
(148) Maes, M.; Kubera, M.; Leunis, J. C.; Berk, M. Increased IgA and IgM responses against gut commensals in chronic depression: further evidence for increased bacterial translocation or leaky gut. *J. Affective Disord.* **2012**, *141* (1), 55−62.
(149) Maes, M.; Twisk, F. N. M.; Kubera, M.; Ringel, K.; Leunis, J.-C.; Geffard, M. Increased IgA responses to the LPS of commensal bacteria is associated with inflammation and activation of cell-mediated immunity in chronic fatigue syndrome. *J. Affective Disord.* **2012**, *136* (3), 909−917.
(150) O'Connor, J.; Lawson, M.; Andre, C.; Moreau, M.; Lestage, J.; Castanon, N.; Kelley, K.; Dantzer, R. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Mol. Psychiatry* **2009**, *14* (5), 511−522.
(151) Zhao, J.; Bi, W.; Xiao, S.; Lan, X.; Cheng, X.; Zhang, J.; Lu, D.; Wei, W.; Wang, Y.; Li, H.; Fu, Y.; Zhu, L. Neuroinflammation induced by lipopolysaccharide causes cognitive impairment in mice. *Sci. Rep.* **2019**, *9*, 5790 DOI: 10.1038/s41598-019-42286-8.
(152) Arentsen, T.; Qian, Y.; Gkotzis, S.; Femenia, T.; Wang, T.; Udekwu, K.; Forssberg, H.; Diaz Heijtz, R. The bacterial peptidoglycan-sensing molecule Pglyrp2 modulates brain development and behavior. *Mol. Psychiatry* **2017**, *22* (2), 257−266.
(153) Tosoni, G.; Conti, M.; Diaz Heijtz, R. Bacterial peptidoglycans as novel signaling molecules from microbiota to brain. *Curr. Opin. Pharmacol.* **2019**, *48*, 107−113.
(154) Guo, H.; Gibson, S. A.; Ting, J. P. Y. Gut microbiota, NLR proteins, and intestinal homeostasis. *J. Exp. Med.* **2020**, *217* (10), e20181832 DOI: 10.1084/jem.20181832.
(155) Pusceddu, M. M.; Barboza, M.; Keogh, C. E.; Schneider, M.; Stokes, P.; Sladek, J. A.; Kim, H. J. D.; Torres-Fuentes, C.; Goldfild, L. R.; Gillis, S. E.; Brust-Mascher, I.; Rabasa, G.; Wong, K. A.; Lebrilla, C.; Byndloss, M. X.; Maisonneuve, C.; Bäumler, A. J.; Philpott, D. J.; Ferrero, R. L.; Barrett, K. E.; Reardon, C.; Gareau, M. G. Nod-like receptors are critical for gut-brain axis signalling in mice. *J. Physiol.* **2019**, *597* (24), 5777−5797.
(156) Humann, J.; Mann, B.; Gao, G.; Moresco, P.; Ramahi, J.; Loh, L. N.; Farr, A.; Hu, Y.; Durick-Eder, K.; Fillon, S. A.; Smeyne, R. J.; Tuomanen, E. I. Bacterial Peptidoglycan Traverses the Placenta to Induce Fetal Neuroproliferation and Aberrant Postnatal Behavior. *Cell Host Microbe* **2016**, *19* (3), 388−99.
(157) Gonzalez-Santana, A.; Diaz Heijtz, R. Bacterial Peptidoglycans from Microbiota in Neurodevelopment and Behavior. *Trends Mol. Med.* **2020**, *26* (8), 729−743.
(158) Murphy, M. P.; LeVine, H., 3rd Alzheimer's disease and the amyloid-beta peptide. *J. Alzheimer's Dis.* **2010**, *19* (1), 311−323.
(159) Stefanis, L. α-Synuclein in Parkinson's disease. *Cold Spring Harbor Perspect. Med.* **2012**, *2* (2), a009399−a009399.
(160) Friedland, R. P. Mechanisms of molecular mimicry involving the microbiota in neurodegeneration. *J. Alzheimer's Dis.* **2015**, *45* (2), 349−62.
(161) Cryan, J. F.; O'Riordan, K. J.; Sandhu, K.; Peterson, V.; Dinan, T. G. The gut microbiome in neurological disorders. *Lancet Neurol.* **2020**, *19* (2), 179−194.
(162) Kim, S.; Kwon, S. H.; Kam, T. I.; Panicker, N.; Karuppagounder, S. S.; Lee, S.; Lee, J. H.; Kim, W. R.; Kook, M.; Foss, C. A.; Shen, C.; Lee, H.; Kulkarni, S.; Pasricha, P. J.; Lee, G.; Pomper, M. G.; Dawson, V. L.; Dawson, T. M.; Ko, H. S. Transneuronal Propagation of Pathologic α-Synuclein from the Gut to the Brain Models Parkinson's Disease. *Neuron* **2019**, *103* (4), 627−641.e7.
(163) Svensson, E.; Horváth-Puhó, E.; Thomsen, R. W.; Djurhuus, J. C.; Pedersen, L.; Borghammer, P.; Sørensen, H. T. Vagotomy and subsequent risk of Parkinson's disease. *Ann. Neurol.* **2015**, *78* (4), 522−9.
(164) Liu, B.; Fang, F.; Pedersen, N. L.; Tillander, A.; Ludvigsson, J. F.; Ekbom, A.; Svenningsson, P.; Chen, H.; Wirdefeldt, K. Vagotomy and Parkinson disease: A Swedish register-based matched-cohort study. *Neurology* **2017**, *88* (21), 1996−2002.
(165) Fitzgerald, E.; Murphy, S.; Martinson, H. A. Alpha-Synuclein Pathology and the Role of the Microbiota in Parkinson's Disease. *Front. Neurosci.* **2019**, *13*, 369−369.
(166) Psichas, A.; Sleeth, M. L.; Murphy, K. G.; Brooks, L.; Bewick, G. A.; Hanyaloglu, A. C.; Ghatei, M. A.; Bloom, S. R.; Frost, G. The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *Int. J. Obes.* **2015**, *39* (3), 424−9.
(167) Hughes, D. T.; Sperandio, V. Inter-kingdom signalling: communication between bacteria and their hosts. *Nat. Rev. Microbiol.* **2008**, *6* (2), 111−20.
(168) Xue, J.; Chi, L.; Tu, P.; Lai, Y.; Liu, C. W.; Ru, H.; Lu, K. Detection of gut microbiota and pathogen produced N-acyl homoserine in host circulation and tissues. *NPJ. Biofilms Microbiomes* **2021**, *7* (1), 53.
(169) Ridlon, J. M.; Ikegawa, S.; Alves, J. M.; Zhou, B.; Kobayashi, A.; Iida, T.; Mitamura, K.; Tanabe, G.; Serrano, M.; De Guzman, A.; Cooper, P.; Buck, G. A.; Hylemon, P. B. Clostridium scindens: a human gut microbe with a high potential to convert glucocorticoids into androgens. *J. Lipid Res.* **2013**, *54* (9), 2437−49.
(170) Belik, J.; Shifrin, Y.; Arning, E.; Bottiglieri, T.; Pan, J.; Daigneault, M. C.; Allen-Vercoe, E. Intestinal microbiota as a tetrahydrobiopterin exogenous source in hph-1 mice. *Sci. Rep.* **2017**, *7*, 39854.
(171) Kau, A. L.; Ahern, P. P.; Griffin, N. W.; Goodman, A. L.; Gordon, J. I. Human nutrition, the gut microbiome and the immune system. *Nature* **2011**, *474*, 327.
(172) Bienenstock, J.; Collins, S. 99th Dahlem Conference on Infection, Inflammation and Chronic Inflammatory Disorders: Psycho-neuroimmunology and the intestinal microbiota: clinical observations and basic mechanisms. *Clin. Exp. Immunol.* **2010**, *160* (1), 85−91.
(173) Mudd, A. T.; Berding, K.; Wang, M.; Donovan, S. M.; Dilger, R. N. Serum cortisol mediates the relationship between fecal Ruminococcus and brain N-acetylaspartate in the young pig. *Gut Microbes* **2017**, *8* (6), 589−600.
(174) Naveed, M.; Zhou, Q. G.; Xu, C.; Taleb, A.; Meng, F.; Ahmed, B.; Zhang, Y.; Fukunaga, K.; Han, F. Gut-brain axis: A matter of concern in neuropsychiatric disorders…! *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2021**, *104*, 110051.
(175) Sun, J.; Li, H.; Jin, Y.; Yu, J.; Mao, S.; Su, K. P.; Ling, Z.; Liu, J. Probiotic Clostridium butyricum ameliorated motor deficits in a mouse model of Parkinson's disease via gut microbiota-GLP-1 pathway. *Brain, Behav., Immun.* **2021**, *91*, 703−715.
(176) Generoso, J. S.; Giridharan, V. V.; Lee, J.; Macedo, D.; Barichello, T. The role of the microbiota-gut-brain axis in neuro-psychiatric disorders. *Braz J. Psychiatry* **2021**, *43* (3), 293−305.
(177) 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.; Redinbo, M. R. Alleviating Cancer Drug Toxicity by Inhibiting a Bacterial Enzyme. *Science* **2010**, *330* (6005), 831−835.
(178) Valdes, A. M.; Walter, J.; Segal, E.; Spector, T. D. Role of the gut microbiota in nutrition and health. *BMJ.* **2018**, *361*, k2179.
(179) Olson, C. A.; Vuong, H. E.; Yano, J. M.; Liang, Q. Y.; Nusbaum, D. J.; Hsiao, E. Y. The Gut Microbiota Mediates the Anti-Seizure Effects of the Ketogenic Diet. *Cell* **2018**, *173* (7), 1728−1741.e13.
(180) Zhang, P.; Wu, X.; Liang, S.; Shao, X.; Wang, Q.; Chen, R.; Zhu, W.; Shao, C.; Jin, F.; Jia, C. A dynamic mouse peptidome landscape reveals probiotic modulation of the gut-brain axis. *Sci. Signaling* **2020**, *13* (642), n/a DOI: 10.1126/scisignal.abb0443.
(181) Gao, B.; Bian, X.; Mahbub, R.; Lu, K. Sex-Specific Effects of Organophosphate Diazinon on the Gut Microbiome and Its

Metabolic Functions. *Environ. Health Perspect.* **2017**, *125* (2), 198−206.
(182) Ni, Y.; Hu, L.; Yang, S.; Ni, L.; Ma, L.; Zhao, Y.; Zheng, A.; Jin, Y.; Fu, Z. Bisphenol A impairs cognitive function and 5-HT metabolism in adult male mice by modulating the microbiota-gut-brain axis. *Chemosphere* **2021**, *282*, 130952.
(183) Diao, J.; Xia, Y.; Jiang, X.; Qiu, J.; Cheng, S.; Su, J.; Duan, X.; Gao, M.; Qin, X.; Zhang, J.; Fan, J.; Zou, Z.; Chen, C. Silicon dioxide nanoparticles induced neurobehavioral impairments by disrupting microbiota−gut−brain axis. *J. Nanobiotechnol.* **2021**, *19* (1), 174.
(184) Lyte, J. M.; Proctor, A.; Phillips, G. J.; Lyte, M.; Wannemuehler, M. Altered Schaedler flora mice: A defined microbiota animal model to study the microbiota-gut-brain axis. *Behav. Brain Res.* **2019**, *356*, 221−226.
(185) Hamady, M.; Knight, R. Microbial community profiling for human microbiome projects: Tools, techniques, and challenges. *Genome Res.* **2009**, *19* (7), 1141−1152.
(186) Zimmermann, M.; Zimmermann-Kogadeeva, M.; Wegmann, R.; Goodman, A. L. Separating host and microbiome contributions to drug pharmacokinetics and toxicity. *Science* **2019**, *363* (6427), eaat9931.
(187) Vernocchi, P.; Del Chierico, F.; Putignani, L. Gut Microbiota Profiling: Metabolomics Based Approach to Unravel Compounds Affecting Human Health. *Front. Microbiol.* **2016**, *7*, 1144 DOI: 10.3389/fmicb.2016.01144.
(188) Bharti, R.; Grimm, D. G. Current challenges and best-practice protocols for microbiome analysis. *Briefings in bioinformatics* **2021**, *22* (1), 178−193.
(189) Knight, R.; Vrbanac, A.; Taylor, B. C.; Aksenov, A.; Callewaert, C.; Debelius, J.; Gonzalez, A.; Kosciolek, T.; McCall, L. I.; McDonald, D.; Melnik, A. V.; Morton, J. T.; Navas, J.; Quinn, R. A.; Sanders, J. G.; Swafford, A. D.; Thompson, L. R.; Tripathi, A.; Xu, Z. Z.; Zaneveld, J. R.; Zhu, Q.; Caporaso, J. G.; Dorrestein, P. C. Best practices for analysing microbiomes. *Nat. Rev. Microbiol.* **2018**, *16* (7), 410−422.
(190) Nagpal, J.; Cryan, J. F. Microbiota-brain interactions: Moving toward mechanisms in model organisms. *Neuron* **2021**, DOI: 10.1016/j.neuron.2021.09.036.
(191) Walter, J.; Armet, A. M.; Finlay, B. B.; Shanahan, F. Establishing or Exaggerating Causality for the Gut Microbiome: Lessons from Human Microbiota-Associated Rodents. *Cell* **2020**, *180* (2), 221−232.
(192) Normand, R.; Du, W.; Briller, M.; Gaujoux, R.; Starosvetsky, E.; Ziv-Kenet, A.; Shalev-Malul, G.; Tibshirani, R. J.; Shen-Orr, S. S. Found In Translation: a machine learning model for mouse-to-human inference. *Nat. Methods* **2018**, *15* (12), 1067−1073.
(193) Brubaker, D. K.; Proctor, E. A.; Haigis, K. M.; Lauffenburger, D. A. Computational translation of genomic responses from experimental model systems to humans. *PLoS Comput. Biol.* **2019**, *15* (1), e1006286.
(194) Kong, G.; Ellul, S.; Narayana, V. K.; Kanojia, K.; Ha, H. T. T.; Li, S.; Renoir, T.; Cao, K.-A. L.; Hannan, A. J. An integrated metagenomics and metabolomics approach implicates the microbiota-gut-brain axis in the pathogenesis of Huntington's disease. *Neurobiol. Dis.* **2021**, *148*, 105199.
(195) Gilbert, J. A.; Blaser, M. J.; Caporaso, J. G.; Jansson, J. K.; Lynch, S. V.; Knight, R. Current understanding of the human microbiome. *Nat. Med.* **2018**, *24* (4), 392−400.
(196) Wood-Charlson, E. M.; Anubhav; Auberry, D.; Blanco, H.; Borkum, M. I.; Corilo, Y. E.; Davenport, K. W.; Deshpande, S.; Devarakonda, R.; Drake, M.; Duncan, W. D.; Flynn, M. C.; Hays, D.; Hu, B.; Huntemann, M.; Li, P.-E.; Lipton, M.; Lo, C.-C.; Millard, D.; Miller, K.; Piehowski, P. D.; Purvine, S.; Reddy, T. B. K.; Shakya, M.; Sundaramurthi, J. C.; Vangay, P.; Wei, Y.; Wilson, B. E.; Canon, S.; Chain, P. S. G.; Fagnan, K.; Martin, S.; McCue, L. A.; Mungall, C. J.; Mouncey, N. J.; Maxon, M. E.; Eloe-Fadrosh, E. A. The National Microbiome Data Collaborative: enabling microbiome science. *Nat. Rev. Microbiol.* **2020**, *18* (6), 313−314.