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Microbiota–brain interactions: Moving toward mechanisms in model organisms

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Abstract

Changes in the microbiota are associated with alterations in nervous system structure-function and behavior and have been implicated in the etiology of neuropsychiatric and neurodegenerative disorders. Most of these studies have centered on mammalian models due to their phylogenetic proximity to humans. Indeed, the germ-free mouse has been a particularly useful model organism for investigating microbiota-brain interactions. However, microbiota-brain axis research on simpler genetic model organisms with a vast and diverse scientific toolkit (zebrafish, *Drosophila melanogaster,* and *Caenorhabditis elegans*) is now also coming of age. In this review, we summarize the current state of microbiota-brain axis research in rodents and humans, and then we elaborate and discuss recent research on the neurobiological and behavioural effects of the microbiota in the model systems of fish, flies and worms. We propose that a cross-species, holistic and mechanistic approach to unravel the microbiota-brain communication is an essential step toward rational microbiota-based therapeutics to combat brain disorders.

Introduction

…
O chestnut tree, great rooted blossomer,
Are you the leaf, the blossom or the bole?
O body swayed to music, O brightening glance,
How can we know the dancer from the dance?

William Butler Yeats, ("Among School Children", 1926)

The concept of organismic boundaries and identities have enamoured scientists and poets, alike. The Irish poet WB Yeats, who received the Nobel Prize in 1923, while exploring the notions of wholeness and distinctions of self and non-self, asked what defined a chestnut tree – the leaf, or the blossom, or the trunk, and if and how can we know the dancer from the dance? In a similar vein, what constitutes an organismal entity and drives its physiological and behavioural output, in the light of the fact that our physical body is cohabited by trillions of microorganisms – bacteria, archaea, viruses, fungi – we are a 'holobiont' of microbial and human cells and genes (Theis et al., 2016). Staggeringly, we have as many or more microbial cells than human cells in our bodies (Sender et al., 2016) and 99% of the genes in our bodies are microbial, with the microbiome comprising over 10 million genes (Gill *et al.*, 2006; Qin *et al.*, 2010).

All metazoans in the past and present have co-existed and co-evolved with microbes – indeed we live in a microbial world, microbes were here long before human cells (Mcfall-Ngai *et al.*, 2013). With such a strong and long association, the fact that the host physiology can be and is strongly modulated by the microbiota should not be a surprise (Sommer and Bäckhed, 2013), with gut microbiota heavily regulating host immunity (Ansaldo et al., 2021; Fung et al., 2017) and metabolism (Fan and Pedersen, 2020). Perhaps most surprising, is the enormous extent to which the microbiota shape the nervous system structure and function (Cryan *et al.*, 2019). Indeed the fact that 'simple' gut bacteria could influence the 'complex' and distant organ such as the brain has been a complete paradigm shift in neuroscience and biomedicine in general (Mayer et al., 2014). The last two decades have witnessed an explosion of research in this exact domain of microbiota-host interactions, where the commensal bacteria have been shown to alter the host's neurophysiology, leading to changes in mood and behavior, including disorders of depression, anxiety and of social behavior (Collins et al., 2012; Cryan and Dinan, 2012; Fang et al., 2020; Morais et al., 2020a; Sharon et al., 2016; Vuong et al., 2017). The majority of this research has been carried out in rodent models, with recent trials in human subjects, fueled by substantial advances in sequencing, bioinformatics and gnotobiotic technologies. Still, many of the mechanisms involved remain poorly deconvoluted.

The development of rational microbiota-based therapeutics to meet the challenges of the neurological diseases requires a comprehensive understanding of mechanisms by which microbes influence the host nervous system, and of processes governing the microbiota assembly in the host. While studies in rodents provide proximity to human-relevant biology, traditional genetic model systems - zebrafish, *Drosophila melanogaster* and *Caenorhabditis elegans,* which enjoy exceptional neurogenetics research tools as well as possess simple microbiota and rich

behavioural repertoire, provide ideal complement to the rodents to realize the true potential of mechanistic microbiome - brain science (Burne *et al.*, 2011; Douglas, 2019). The synergy between these 'simple' models and rodent and human research can provide the much-needed tractability and translation to promote this exciting field forward (**Figure 1**). In this review, we will first discuss pathways of microbiota-gut-brain axis communication and general principles distilled from recent rodent and human literature, before moving on to elaborating the research on microbial modulation of nervous system in zebrafish, flies and worms.

Microbiota-Gut-Brain Axis - Pathways of Communication

Although the gut-brain interactions in the context of digestive function and satiety have been known for a long time (Berthoud, 2008), research in the last two decades has increasingly incorporated the various aspects of microbiota into the gut-brain axis and focused on cognitive and psychological effects of this communication. The gut microbiota employs multiple channels of communication to influence the brain and behaviour. The most prominent of these pathways include vagus nerve activation, generation of circulating metabolites of microbial origin, stimulation of immune pathways, stimulation of enteroendocrine cells and enteric nervous system signaling and the generation of neurotransmitters in the gut itself. Each of these signaling routes have been the focus of individual recent reviews covering many recent discoveries about the dynamic molecular cross-talk between gut microbes and the nervous system, though some are yet to be extensively validated (Fülling et al., 2019; Fung, 2020; Liu and Forsythe, 2021; Needham et al., 2020; Strandwitz, 2018). In the subsequent sections, we have highlighted representative studies elucidating these different pathways in the context of microbiota-gut-brain communication in different model systems. Particular emphasis in the last years has been on the discovery and elucidating the role of microbially produced and/or induced metabolites in relaying the microbial signals to the brain (**Figure 2**): short-chain fatty acids (SCFAs), amino-acid derivatives (catecholamines and other indole derivatives, p-cresol), and secondary bile-acids (Needham et al., 2020; Swann et al., 2020). With the current state of the field comprising mainly of correlative studies of gut metabolome associations with neurological conditions, the therapeutic translation of microbial metabolites would require more studies focused on investigating the effects of these molecules on brain cells, brain activity and behaviour. An important target that is emerging to mediate effects of bioactive microbial metabolites on host physiology are the G-protein coupled receptors (GPCRs), including many orphan GPCRs (Chen et al., 2019a; Cohen et al., 2017; Colosimo et al., 2019). Metabolites produced by the human microbiota have been shown to act as agonists for a wide range of GPCRs. SCFAs, primarily produced by gut-microbial fermentation of host-indigestible fibers and having widespread effects on gut-brain communication, were shown to activate the orphan GPCRs GPR41 and 43 (Brown et al., 2003; Silva et al., 2020). Commensal supernatants from *Morganella morganii* activated both dopamine and histamine receptors whereas from two *Lactobacillus reuteri* strains activated histamine receptors (Chen et al., 2019a). Bacterially produced aromatic amines such as tryptamine and tyramine were shown to agonize serotonergic and dopaminergic GPCRs, respectively (Colosimo et al., 2019). Nicotinic acid, isolated from the cultures of gut commensals *Lactobacillus plantarum* and *Ruminococcus gnavus,* is a ligand for hydroxycarboxylic acid receptor GPR109A, a receptor found to be

significantly upregulated in the substantia nigra of Parkinson Disease patients(Colosimo et al., 2019; Wakade et al., 2014). Going forward, microbial metabolome screening will serve as an important tool to both identify gut commensal microbes with effects on host physiology as well as de-orphanize GPCRs.

Microbiota-Gut-Brain Axis - Mammals

The use of rodents as model organisms has transformed our understanding of human nervous system in health and disease. Specifically, the rodent models mimicking the underlying genetic cause or phenotypic outcome in case of several neurobehavioural disorders have yielded invaluable insights into the etiology of these diseases (Nestler and Hyman, 2010). From the perspective of microbiota-led effects on host, the germ-free mouse has yielded some of the most compelling evidence for a role of microbiota in gut-brain communication. Major results with GF mice show that microbiota is essential for appropriate stress response, anxiety-like behaviors, as well as social behaviour (Luczynski et al., 2016). GF mice also provide a clean background onto which defined specific bacteria can be introduced from variety of host backgrounds as well as at various points of host development, allowing a thorough examination of microbiota-driven disease outcomes and critical windows of host-microbiota development. Finally, transfer of the gut microbiota associated with the disease via fecal microbiota transplant (FMT) in GF rodents and subsequent phenotypic analysis is also widely used to imply causality (Gheorghe et al., 2021; Walter et al., 2020). Furthermore, the rodent microbiota though distinct, bears the closest resemblance to the human microbiome among the traditional model systems, making them ideal for translation studies (Hugenholtz and de Vos, 2018).

Perturbations in the gut microbiota have been shown to impact varied aspects of neurophysiology and behaviour. Processes impacted by the state of gut microbiota include neurogenesis, synaptic plasticity, neurotransmitter signaling, neuronal morphology, neuroinflammation, neurodevelopment, and neurochemistry across regions of hippocampus, amygdala, prefrontal cortex, and hypothalamus (Cryan et al., 2019). Moreover, there is mounting evidence linking microbiota alteration to neurobehavioural diseases spanning neurodevelopmental disorders (autism spectrum disorder [ASD] and schizophrenia), neurodegenerative diseases (Alzheimer's disease [AD], Parkinson's disease [PD], and multiple sclerosis [MS], and mood disorders (depression and anxiety). A multitude of reviews have been written in this fast-paced field over the past few years (Cryan and Dinan, 2012; Sampson and Mazmanian, 2015; Sharon *et al.*, 2016; Vuong *et al.*, 2017) and we guide the readers to very recent ones (Cryan et al., 2019, 2020; Morais et al., 2020a) for the most-up-to-date and elaborate overview of the rodent and human literature. Most of the evidence illustrating microbial effects on brain physiology has emerged from mouse models lacking microbiota, germ-free and antibiotic-treated mice, in addition to a growing number of studies using probiotics and prebiotics to influence microbiota structure-function. In the following subsections, we highlight studies that aim at mechanistically linking gut microbiota to neurobehavioural disorders in preclinical rodent models (also see **Table 1** for selected examples with a special focus on disease outcomes and emerging mechanisms).

Microbiota and Brain – Rodent Models Relevant to Psychiatry

While it is increasingly clear that microbiota plays a strong associative role in the aetiology of a range of mood and neurodevelopmental disorders in animals, for some disorders such as depression and autism spectrum disorder (ASD), the link is more evident and deterministic.

Depression and anxiety

The dysregulated immune response, associated with anxiety and depression, intersects strongly with the state of gut microbiota towards contributing the etiology of these disorders (Cruz-Pereira et al., 2020). Gut microbiota regulate the proportion of pro-inflammatory T helper 17 cells (Th17) and anti-inflammatory Tregs in the gastrointestinal tract, which is essential to maintain immune homeostasis and prevent inflammation (Fung, 2020). A recent study described a novel signaling mechanism by which depressive-like behaviours in mice are regulated by the microbiome in a Th17 cell-mediated manner (Medina-Rodriguez et al., 2020). The authors observed that mice deficient in segmented filamentous bacteria (SFB) were resilient to the induction of learned helplessness, a depressive-like behavior following exposure to foot shock stress protocol. Importantly, gavaging of mice with SFB monocolonized feces resulted in sensitization to learned helplessness. To mechanistically understand how SFB could modulate depressive-like behaviour, the authors explored quorum sensing, a bacterial regulatory process that involves the secretion of autoinducer molecules that shape bacterial population numbers and gene expression. SFB produce a quorum-sensing molecule - autoinducer-2 (AI-2) and promote the host generation of of serum amyloid proteins, which raises Th17 cell count. Th17 cells were necessary to induce depressive-like behaviors by AI-2, as AI-2 administration did not cause depressive-like behaviors or generation of serum amyloid proteins in Th17-deficient mice after stress. Interestingly, Oleic acid, a fatty acid occurring naturally in various plant and animal fats and an AI-2 inhibitor, exhibited antidepressant properties. Oleic acid administration decreased depressive-like behavior, intestinal serum amyloid protein production, and hippocampal Th17 cell numbers.

There is also increasing evidence for altered microbiota leading to depression from FMT studies where the colonization of normal animals with microbiota from depressed patients induced depressive like behaviours (Yang et al., 2020). FMT from patients with depression into microbiota-deficient rats caused anxiety-like behaviour and anhedonia in the sucrose consumption test, as well as changes in tryptophan metabolism (Kelly et al., 2016). Similarly, microbiota transplant from depressed patients into GF mice resulted in increased immobility time in the tail suspension and forced swim tests and alterations in the carbohydrate and amino acid metabolism (Zheng et al., 2016). In another study, naive rats, receiving FMT from vulnerable rats as opposed to resilient ones following chronic social defeat stress, showed higher depression-like behaviour and elevated microglial density and IL-1β expression in the ventral hippocampus, further substantiating the contribution of gut microbiota to depressive-like behaviour and neuroinflammatory processes (Pearson-Leary et al., 2020). In a recent study, FMT from patients of alcohol use disorder to antibiotic-treated mice resulted in increased depressive-like behaviour measured in a forced-swim test and corticosterone levels as well as reduced sociability (Leclercq et al., 2020). This was accompanied by downregulation of myelin-related gene expression in

striatum and frontal cortex as well as significant rise in pro-inflammatory cytokines (Tnfa and IL1b) in the frontal cortex. Notably, levels of ß-hydroxybutyrate (BHB), a liver-generated ketone body utilized as energy substrate by neurons, were significantly decreased in mice receiving the FMT. Ketogenic diet, known to enhance the levels of BHB, was found to improve sociability and increase myelin-related gene expression.

Another mechanism by which gut microbiota may be communicating with the brain is through the vagus nerve (Fülling et al., 2019). An intact vagus nerve has been shown to be critical to relay the anxiolytic effects of probiotic strains including *Lactobacillus rhamnosus* (JB-1) and *Bifidobacterium longum* (Bercik et al., 2011a; Bharwani et al., 2020; Bravo et al., 2011) as well as for cognitive and electrophysiological effects of a prebiotic 2´-fucosyllactose,an abundant human milk oligosaccharide (Vazquez et al., 2016). Vagotomy has been shown to decrease brain-derived neurotrophic factor (BDNF) expression and neurogenesis in hippocampus (O'Leary et al., 2018), which are also known to be altered in GF and antibiotic-treated mice, further supporting the key role of vagus nerve as the conduit between gut microbiota and brain (Bercik et al., 2011b; Clarke et al., 2013; Diaz Heijtz et al., 2011; Neufeld et al., 2011; Sudo et al., 2004). Concentrations of microbial metabolites like tryptophan indole-metabolites (indolelactate and indolepropionate) and bile acids have also been identified to inversely correlate with pro-depression microbiota component in a model of high-fat diet induced depressive like behaviour in rats (Abildgaard et al., 2021). A gut commensal species, *Morganella morganii*, has been demonstrated to convert the essential amino acid L-Phe into phenethylamine (PEA), a potent psychoactive agent, that in mono-colonized mice traverses across the blood-brain barrier and causes phenethylamine poisoning upon administration of monoamine oxidase inhibitor (MAOI) (Chen et al., 2019a). Since MAOIs constitute a major treatment alternative for depressed patients, inter-individual variability in PEA produced by gut microbes could potentially explain the variable efficacy of MAOIs on depression.

Autism-spectrum disorder

Interdependent communication among the microbiota, gut function, immune system and behavior has been observed in the context of ASD (Cryan et al., 2019). The microbiota-immune axis has also been implicated in ASD models. In a maternal immune activation model of ASD, SFB and Th17-inducing gut microbes were found to induce aberrant cortical neurodevelopment in the fetal brain and behavioural dysfunction in the progeny via release of cytokine IL-17a (Choi et al., 2016a; Kim et al., 2017). A number of studies have found significant differences in the composition of gut microbiota of ASD mouse models, ranging from genetic (*Shank3B*$^{-/-}$ *and Cntnap2*$^{-/-}$) to environmental (maternal immune activation, valproic acid administration, and chronic high-fat diet during pregnancy) to idiopathic (BTBR) ASD models (Buffington et al., 2016, 2021; Golubeva et al., 2017; Hsiao et al., 2013; Sgritta et al., 2019) (see **Table 1**). Supplementation of specific bacterial strains *L. reuteri* and *B. fragilis* have been shown to reverse social deficits and anxiety-like and stereotyped behavior. The rescue of social deficits by *L. reuteri* were not mediated by restoring the gut microbiome composition in the *Shank3B*$^{-/-}$ mice. Instead, *L. reuteri* was shown to rescue social interaction-induced synaptic plasticity in the dopaminergic ventral tegmental area (VTA) of *Shank3B*$^{-/-}$ ASD mice. This rescue was dependent on the vagus nerve and was not

observed in mice that lacked the oxytocin receptor in dopaminergic neurons (Sgritta et al., 2019). Moreover, *L. reuteri* administration restored the number of oxytocin-positive neurons in the paraventricular nucleus (PVN) of the hypothalamus of *Shank3B*$^{-/-}$ mice and oxytocin administration was found to rescue the social deficits in vagotomized mice. In the *Cntnap2*$^{-/-}$ mouse model of ASD, interestingly, the hyperactivity phenotype was found to be caused by host genetics, while the social behaviour phenotype was exclusively mediated by the gut microbiome (Buffington et al., 2021). *L. reuteri* reversed the deficits in oxytocin-producing neuron count, social deficits and the social-interaction induced synaptic plasticity in the VTA dopaminergic neurons, but not the hyperactivity phenotype. The reversal of deficits was, in part, found to be modulated by the endogenous levels of a tetrahydrobiotperin, a microbially-induced metabolite in the gut of the *Cntnap2*$^{-/-}$ mice. This study widens the brain-centric understanding of genetic neurological disorders, where a genetic mutation and gut microbiome can interdependently lead to behavioural abnormalities. More microbially produced or induced metabolites such as p-cresol are also now under investigation in the etiology of ASD (Bermudez-Martin et al., 2021). SCFAs, a prominent class of microbial metabolites, are also critical for neurodevelopmental processes including maturation of microglia (Erny et al., 2015; Liu et al., 2021) and have been shown to restore maternal obesity-induced cognitive and social behavioral deficits in offspring via promoting microglia-neuron communication and synaptic function in hippocampus and prefrontal cortex of offspring (Liu et al., 2021).

Early-life neurodevelopment

Furthermore, the microbiota is dynamic and plastic, acquired at birth, but strongly influenced by our environment and our lifestyles, and even has been seen as a reflection of our “personal historical ecosystems” (Gordon and Klaenhammer, 2011). The very fact that the microbiome, unlike our inherited human genome, is readily malleable, provides an extremely attractive vantage point to alter our neurophysiology from a therapeutic point of view. Some of the common factors known to impact the microbiota-gut-brain activity are diet, exercise, medications, and mode of delivery at birth (Ratsika et al., 2021). It has recently been reported that Caesarian-section (CS) has enduring neurobehavioural effects in mice including social deficits, which can be partly reversed using supplementation from birth with a *Bifidobacterium breve* bacterial strain that is selectively depleted in early-life upon CS, or with a dietary prebiotic mixture (Morais et al., 2020b). The role of early-life microbiota in neurobehavioral development has further been looked at using GF models and antibiotic administration in the critical periods of development, with studies converging on strikingly similar deficits in social behaviour in adulthood hinting at commonality in mechanisms (Desbonnet et al., 2014; Leclercq et al., 2017; O’Connor et al., 2021). A recent mechanistic report shows depletion of maternal gut microbiota has strong influence on fetal neurodevelopment in mice, including causing deficits in thalamocortical axonogensis and somatosensory behaviour, which could partly be restored using select microbiota-dependent metabolites (trimethylamine-N-oxide, 5-aminovalerate, imidazole propionate, and hippurate) (Vuong et al., 2020). Other recent studies demonstrating remarkable translation of results obtained from preclinical gnotobiotic animal models to humans, utilized mice and pig models to design microbiota-directed foods aimed at alleviating neurodevelopmental and growth deficits in undernourished children (Chen et al., 2021; Gehrig et al., 2019)

A factor that has been understudied in the context of microbiota-gut-brain studies is sex, given it is an important variable in the development of many of the neuropsychiatric disorders, with anxiety and depression more prevalent in females and ASD and schizophrenia more common in males, likely due to differences in sex steroid hormones and genetics (McCarthy et al., 2017). Since sex can influence gut microbiota composition and diversity, and reciprocally microbiota can regulate sex steroid hormone conjugation processes, many neurobehavioural processes linked with the neuropsychiatric disorders should be viewed from the lens of complex microbiota-sex-brain interactions (Jaggar et al., 2020).

Microbiota and Brain – Rodent Models Relevant to Neurology

There is an increasing attention on the role of gut microbiota in the onset of neurological disorders such as Alzheimer's Disease (AD), Parkinson's Disease (PD) and Multiple Sclerosis (MS). Transgenic AD mouse models, that are GF or treated with antibiotics, did not develop plaques (Harach et al., 2017; Minter et al., 2016) and FMT from wildtype mice improved cognitive deficits. Similarly for PD and MS, the key neurobehavioural symptoms were partly recapitulated upon FMT from patients in rodents (Berer et al., 2017; Sampson et al., 2016) and microbiome of the animal models, phenocopying the characteristic symptoms of these diseases, has been well-characterized (see **Table 1**). Microbiota members from *Akkermansia and Lachnospiraceae* have been implicated in multiple studies to play a key role. In a recent study, contrary to the view that elevated *Akkermansia* in MS has a detrimental role, the authors showed amelioration of symptoms in a mouse model of MS upon colonization with MS-patient derived *Akkermansia*, which was associated with a reduction in IL-17–producing T cells (Cox et al., 2021). This further demonstrates that in this fast-evolving field, many mechanisms by which the microbiome affects the etiology of neurological disorders are not well defined, and several confounding factors exist that need further elucidation. This would be beneficial for future microbiome studies with AD, PD and MS as well as with Amyotrophic lateral sclerosis (ALS) and Huntington's disease, where the association with the state of gut microbiota is also emerging. Another interesting avenue where gut microbiota can have a role to play is in the bioavailability of therapeutic drugs targeted to neurological disorders, as has been shown for Levodopa (L-dopa) and PD (Maini Rekdal et al., 2019). It was demonstrated that a tyrosine decarboxylase from *Enterococcus faecalis* first converted L-dopa to dopamine*,* which was subsequently transformed to m-tyramine by a dehydroxylase from *Eggerthella lenta.* Moreover, a compound, α-fluoromethyltyrosine, was identified to inhibit microbial L-dopa decarboxylation in PD patients' microbiota and increase bioavailability of L-dopa in mice. Another neurological disorder where gut microbiota is coming up as a key player is epilepsy. For over a century, a ketogenic diet has been known to reduce the seizures in patients and is a particularly effective treatment strategy for individuals who do not respond to existing anti-epileptic drugs. In animal models of epilepsy, it was demonstrated that depletion of the gut microbiota abrogated the protective effects of the ketogenic diet and promoting the ketogenic diet-associated microbiota in mice conferred significant seizure protection (Olson et al., 2018).

Microbiota and Brain – Human studies

Building on the pre-clinical studies, there is increasing evidence from cross-sectional clinical studies in humans substantiating the notion of altered microbial composition contributing to the pathophysiology of neurological and mental health disorders, including AD, PD, MS, ASD and stroke (Cryan et al., 2020). Analysing the microbiome composition in a large human cohort, a recent study reported reduction in the butyrate-producing bacteria *Faecalibacterium* and *Coprococcus* in patients with depression, and devised an analytical framework wherein the neuroactive potential of sequenced microbiome was described (Valles-Colomer et al., 2019). Using such approaches, key microbiota members are being identified as important susceptibility factors for these disorders and FMT from patients to GF rodents is pushing the translatability forward. However, most studies to date are underpowered, often with participant-selection bias, and provide a snap-shot assessment, requiring that caution is exercised to avoid overinterpreting such data. Moving forward, longitudinal cohort studies and randomized controlled trials in humans are essential to bolster the concept. Moreover, variables that directly impact gut microbiota composition such as diet, medication and exercise need to be accounted for while interpreting the data emanating from these clinical studies.

Zebrafish (Danio rerio): Utility as a model organism for microbiota – brain communication.

The same advantages that propelled the zebrafish to the ranks of premier model organisms are now being leveraged to explore host-microbiota interactions: its fast external development, high reproductive output, and optical transparency that enables *in-vivo* imaging of physiological processes. Particularly, the *ex-utero* development that allows for easy manipulation of microbial exposure and to study the microbial influence throughout development, as well as the neuroanatomical similarities with mammals, make zebrafish an ideal system to investigate the role of the microbiota in nervous system development and function. The zebrafish possess an impressive range of behavioural repertoire, including social, cognitive, sleep, stress and anxiety behaviour (Fontana et al., 2021; Lyons and Rihel, 2020; Stewart et al., 2015). There is a suite of tools and resources available to carry out precise genetic manipulations (Li *et al.*, 2016) and to record and manipulate neuronal activity at a cellular resolution throughout the larval whole brain (Vanwalleghem et al., 2018).

Furthermore, relatively simple, inexpensive, and scalable gnotobiotic methods have been developed to obtain and raise GF zebrafish leveraging high fecundity of zebrafish (Melancon et al., 2017; Pham et al., 2008). Most GF zebrafish studies have focused on early larval stages up to 7 to 9 days post-fertilization (dpf), after which it becomes costly and labour intensive to raise them to adulthood in an axenic environment, owing to strict requirements for sterilized food and housing conditions (Pham *et al.*, 2008). 16S rRNA based sequencing studies of bacterial communities in the zebrafish gut have revealed a core microbiota comprising of γ-Proteobacteria and Fusobacteria classes (Roeselers et al., 2011). Despite the disparities in taxonomic composition, there is a substantial homology in the functional categories encoded in the zebrafish

gut microbiome to those encoded in the human and mouse gut microbiomes, suggesting that these microbiomes may operate similarly (Gaulke *et al.*, 2020). Interestingly, in a reciprocal gut microbiota transplant experiments between GF zebrafish and mice, the host gut and associated factors were found to play a more predominant role in sculpting the bacterial communities, and the microbiota from zebrafish and mouse could elicit similar responses in zebrafish (Rawls *et al.*, 2006). This suggests, despite distinct microbial communities, the microbial stimuli may be shared. Furthermore, a simplified human microbiota has also been shown to successfully colonize GF zebrafish larvae, substantiating the case for the use of zebrafish as a model to study human gut microbiota members in mono-association or consortia (Arias-Jayo *et al.*, 2018).

Effects of microbiota manipulations on zebrafish brain and behaviour.

The majority of the studies linking the microbiota to brain and behaviour in zebrafish have utilized specific bacterial strains (probiotics) and antibiotics to alter the microbiota and reported changes in stress-related and social behaviour (Borrelli et al., 2016; Davis et al., 2016b, 2016a; Schneider et al., 2016; Valcarce et al., 2020; Wang et al., 2016).

Adult zebrafish, after being administered with the probiotic *Lactobacillus rhamnosus* IMC 501 for 28 days in the diet, showed differences in shoaling and exploratory behaviour (Borrelli *et al.*, 2016). Also, the probiotic-fed fish had elevated expression and altered promoter DNA methylation profiles of brain-derived neurotrophic factor (BDNF) as well as of key genes involved in serotonergic neurotransmission in the brain (Cuomo et al., 2021), supporting existing literature connecting microbiota and these systems in mice (Clarke et al., 2013; Neufeld et al., 2011). Administration of *Lactobacillus plantarum* to adult zebrafish significantly reduced anxiety-related behaviour as assayed in the novel tank test and altered GABAergic and serotonergic signaling in the brain (Davis et al., 2016b). Also, *L. plantarum* attenuated the stress-induced shifts in the gut microbiota composition in adult zebrafish. Notably, the major core phylum (Fusobacteria) which was significantly reduced in zebrafish under chronic stress, remained intact in *L. plantarum* treated fish. However, the physiological responses such as cortisol levels and blood cell counts remained unaffected in *L. plantarum* treated chronically stressed fish (Davis et al., 2016b). Another study showed that 4-month long supplementation of *Lactobacillus rhamnosus* CECT8361 and *Bifidobacterium longum* CECT7347 significantly reduced bottom-dwelling behaviour of adult zebrafish in the novel tank test, which could be correlated to a lower state of anxiety (Valcarce *et al.*, 2020). However, administration of another bacterial strain, *Lactobacillus rhamnosus* GG (LGG) was found not to alter the swimming behaviour of adult zebrafish in the novel tank test (Schneider *et al.*, 2016), and the anxiolytic effects of ethanol exposure were also not significantly altered by LGG intake.

Complementing the studies with probiotic strains, antibiotics have also been used to explore the role of microbiota in regulating behaviour. Adult zebrafish exposed to a mixture of β-diketone antibiotics at lower concentrations showed an increase in the exploratory behaviour (Wang *et al.*, 2016). This may be interpreted as an indicator of anxiolytic behaviour, which is in contrast with the exposure to higher concentrations, where it led to anxiety-like behaviour. This trend was mirrored in social behaviour assay where the antibiotics at low dosage increased the shoaling

behaviour in the zebrafish, although high concentration decreased the social cohesion (Wang *et al.*, 2016).

The adult zebrafish, though developmentally mature, is not readily amenable for gnotobiotic studies. The GF zebrafish larvae, alternatively, are easier to obtain and maintain, and have been used to study the role of microbiota in neurobehavioral development and stress responses (Davis et al., 2016a; Phelps et al., 2017). The absence of microbiota increased the locomotor activity in 6 dpf (Davis et al., 2016a) and in 10 dpf GF zebrafish larvae (Phelps *et al.*, 2017). This is congruent to the previous findings in mice, where GF mice exhibit increased locomotion in an open-field test as compared to specific-pathogen-free controls (Diaz Heijtz *et al.*, 2011). The hyperactivity in GF larvae was pheno-copied in antibiotic-treated conventionally colonized larvae at 10 dpf (Phelps *et al.*, 2017), demonstrating the role of microbiota in regulating locomotion activity. Furthermore, there appears to be a timing requirement to alter the locomotion responses as larvae conventionalized on 1, 3, or 6 dpf did not exhibit hyperactivity, while conventionalization on 9 dpf failed to block hyperactivity (Phelps *et al.*, 2017). This suggests that the early-life microbiota disruption during specific temporal windows may result in aberrant neurobehavioral development. In addition, mono-colonization of GF larvae on day 1 with *Aeromonas veronii* or *Vibrio cholerae* (representative members of zebrafish microbiota), was able to block the behavioural hyperactivity. However, treatment with heat-killed bacteria and purified microbe-associated molecular patterns were insufficient to prevent the hyperactivity, suggesting the TLR-dependent signalling may not be directly linked to the microbiota-mediated behavioural modulation in zebrafish (Phelps *et al.*, 2017). In another study, microbiota colonization status was also shown to influence metabolism of 17-β estradiol (E2), a key sex steroid involved in endocrine signaling in zebrafish and modify the effects of E2 on swimming behaviour (Catron *et al.*, 2019). This supports the concept of microbiota as a key component in the biotransformation of xenobiotics and other chemicals (Guthrie et al., 2019).

In zebrafish larvae, anxiety-like behaviour is often estimated by measuring thigmotaxis or preference for the outer edge of a chamber during periods of light (Schnörr *et al.*, 2012). While 6 dpf GF larvae displayed reduced thigmotactic behaviour (Davis et al., 2016a), the phenotype was not recapitulated in 6 or 10 dpf GF larvae in another study (Phelps *et al.*, 2017). The inconsistency in the results could be attributed to different strains as well as variations in housing and experimental lighting conditions used in the two studies. The reduced anxiety in GF larvae is similar to previous rodent work, where GF mice showed reduced anxiety-related behaviour as compared to conventionally raised mice (Clarke et al., 2013; Diaz Heijtz et al., 2011; Huo et al., 2017; Neufeld et al., 2011). However, opposing data also exists where GF mice of particular inbred strains showed elevated anxiety-like behaviour (Bercik et al., 2011b). Additionally, GF zebrafish larvae showed a significantly lower cortisol response to an acute osmotic stressor as compared to conventionally raised larvae. This is inconsistent with previous work in mice which demonstrates that the GF mice have elevated corticosterone levels upon restraint stress (Sudo *et al.*, 2004). The discrepancies may be attributed to the genetic confounds since behavioural phenotypes can be dictated whether the experiments were conducted in outbred or inbred strains of mice, or due to the nature of the type of stressors utilized in each study. Another important source of dissimilarity is the primary output of the HPA axis in fish and rodents: while rodents

produce corticosterone as the primary glucocorticoid during a stress response, the zebrafish produce cortisol, much like the humans (Wendelaar Bonga, 1997).

Another interesting behaviour displayed by zebrafish is the remarkable alarm response upon exposure to conspecifics that are injured. This alteration in affective behaviour, which is comprised of darting and freezing behaviours in locomotion, is elicited by the release of alarm substance (Schreckstoff) from the injured fish. A recent study has found that the alarm substance, which is produced by the club cells in the skin of the fish, also contains a bacterial component. Lysates from a zebrafish Staphylococcus isolate were sufficient to evoke the alarm response, acting along with the host substances in the mucus (Chia *et al.*, 2019). This suggests that the commensal bacteria from one individual could influence the emotional state of the others through social olfactory signalling.

There also has been a body of work highlighting the role of resident microbiota in modulating social behaviours of the host across the animal kingdom (Sherwin *et al.*, 2019). However, the mechanisms linking microbiota to social neurodevelopment are not well understood. A recent study utilized the zebrafish gnotobiotic techniques and external development to show that the microbiota during early life (0 to 7 dpf) was critical for the emergence of social behaviour in 14 dpf larvae (Bruckner *et al.*, 2020). Harnessing the optical transparency of larval zebrafish, the authors found that the microbiota is essential for appropriate arborisation of neuronal morphology in the specific forebrain areas necessary for normal social behaviour. This remodelling of the neurite complexity and targeting was mediated via the microglia, the brain's resident immune cells that regulate the neurite growth and pruning (Bruckner *et al.*, 2020). Moreover, the authors observed that the microbiota promoted the infiltration and hence abundance of the microglia in the forebrain, though there was no effect on the microglial activity. The study further underscores the concept of critical windows of early neurodevelopment as being readily amenable to microbiota modulation, which can strongly affect behaviours that manifest at a later developmental stage which has been widely studied in rodents and humans (Cowan et al., 2020).

The use of larval zebrafish is also pushing the envelope in terms of mechanistic dissection of the microbe-brain signalling. Rawls and colleagues have recently provided compelling evidence for the sensing of bacterial tryptophan metabolites by the enteroendocrine cells (EEC) that leads to the activation of the vagal and enteric neuronal pathways (Ye *et al.*, 2020). The neurons of the enteric nervous system as well as from autonomic nervous system, including sensory neurons from the vagal ganglia innervate the vertebrate intestine. The vagal sensory nerve fibers relay signals from peripheral cues to the central nervous system (CNS) and modulate a variety of brain functions (Fülling et al., 2019). The authors used *in vivo* calcium imaging of the EEC and nervous activity to demonstrate that bacteria *Edwardsiella tarda* and its derived tryptophan catabolites trigger vagal sensory ganglia activation via EEC transient receptor potential ankyrin A1 (Trpa1) signalling. Since the EEC-vagal signaling constitutes an important route of communication between gut microbiota and CNS, it may be envisaged that specific tryptophan metabolites could have the potential to affect CNS processes and behaviour. Another recent paper shed light on the role of short chain fatty acids as anti-inflammatory agents in zebrafish, alike in rodents (Cholan *et al.*, 2020). Considering all the myriad advantages, the zebrafish with a diverse microbiota but

still among the simplest vertebrate models, is poised to be an important model to understand the mechanistic basis of host-microbiota communication moving forward.

The Fruit Fly (*Drosophila melanogaster*): Utility as a model organism for microbiota – brain communication.

Drosophila melanogaster has been the flag bearer model organism for research in behavioural genetics, which spans the last several decades (Sokolowski, 2001). There is a plethora of complex behaviors exhibited by the fly that are amenable to dissection at a genetic and a neural circuit level, owing to a terrific genetic and recent connectomic toolkit available to Drosophila researchers (Hales *et al.*, 2015; Shan Xu *et al.*, 2020). It is not a surprise that drosophila neuroscience has and continues to yield important insights in diverse fields such as biological rhythms, learning and memory, sleep, aggressiveness, sexual behavior, and more (Brembs, 2019). Drosophila has also been used extensively to understand host-pathogen interactions (Buchon et al., 2014). The last few years has seen this simple and genetically tractable organism, providing insights in the domain of microbiota-brain interactions as well. From the microbiota point of view, the fly model provides a definite advantage of possessing a relatively simple microbiota consisting of approximately 20 strains, primarily from the families *Acetobacteraceae* and *Lactobacillaceae* (Chandler *et al.*, 2011). These microbes, being aerotolerant or obligate aerobes, are distinct from the mainly anaerobic commensal gut bacteria found in humans. However, they do offer the advantage that majority of them are amenable to culturing and in-vitro genetic manipulation, greatly facilitating the identification of microbial metabolites that are directly influencing the host physiology, for example insulin mediated larval growth (Shin *et al.*, 2011) and the glucose oxidation (Chaston et al., 2014). Furthermore, the germ free or axenic Drosophila can be produced in large numbers and can be maintained on nutritive media for many generations. Complementing the Drosophila, honey bee, a social insect, is also emerging as a promising model to investigate extended behavioral phenotypes of gut microbes (Liberti and Engel, 2020; Vernier *et al.*, 2020).

Effects of microbiota manipulation on brain and behaviour.

Drosophila thrive on rotten fruits which are teaming with microbes and their metabolites. The interaction of these bacterial organic molecules with the gustatory and olfactory neurons of the fly guide it to nutrient rich food to lay eggs and to avoid pathogenic niches (Itskov and Ribeiro, 2013; Joseph and Carlson, 2015). Fly larvae detect bacterial short-chain fatty acids (propionate and butyrate) using odorant receptors (Or) Or30a and Or94b located on the olfactory sensory neurons, triggering feeding behaviors (Depetris-Chauvin *et al.*, 2017).

In one of the early studies linking host social behavior and microbiota, the authors showed that commensal bacteria influenced mating preference in *Drosophila melanogaster* (Sharon *et al.*, 2010). Depending on the diet on which the fly was raised, they exhibited a mating preference which was maintained for multiple generations. The fly microbiota was identified as a major contributing factor as the antibiotic treatment abolished mating preference, which was further

validated by colonization experiments with *Lactobacillus plantarum*, mixed culture of *Lactobacillus* species, and microbiota prior to antibiotic exposure. Although detailed mechanism by which commensal bacteria can influence mating preference remains to be elucidated, microbiota-led modulation of levels of cuticular hydrocarbon sex pheromones is a potential avenue. In another study, the interplay between an epigenetic modifier, microbiota and fly social behaviours (that mimic ASD symptoms) was explored (Chen et al., 2019b). Flies with reduced levels of histone demethylase KDM5 (whose loss-of-function mutations are linked with ASD in mice and humans) exhibited intestinal barrier impairment and alterations in gut microbiota composition and social behavior, all of which could be partially rescued by feeding a probiotic Lactobacillus strain (*L. plantarum L168*). In a mechanistic work that shows the microbiota can have a causal role in behaviour, the authors found that the walking activity of the *Drosophila melanogaster* can be affected by a specific gut bacterium (Schretter *et al.*, 2018). As compared to conventionally colonized flies, the drosophila raised in axenic conditions displayed hyperactive behaviour. This increase in walking speed and daily activity in germ-free conditions was reversed by colonization with *Lactobacillus brevis*, a gut commensal of fruit flies, but not *Lactobacillus plantarum.* Delving deeper into the mechanism, the study showed that effects on locomotion were caused by changes in the sugar (trehalose) metabolism, which was modulated by the enzyme xylose isomerase from *L. brevis*. Octopamine, the equivalent of noradrenaline in invertebrates, was identified to be the host effector mediating the effect of xylose isomerase on locomotion as exogenous supplementation of octopamine or activation of octopaminergic neurons removed the effects of xylose isomerase treatment. In other recent studies, effects of gut microbiota on aggression in flies were studied. While GF male flies displayed reduction in aggressive behaviour (Jia et al., 2021), antibiotic-treated flies exhibited significantly more aggressive behaviors (Grinberg et al., 2020). The agression in GF flies was restored by microbiota recolonization, with diet and octopamine signaling playing a key role (Jia et al., 2021). Antibiotic-treated flies had elevated levels of vCA and (Z)-9 Tricosene, pheromones related with aggression in flies, as well as increased expression of the pheromone receptors and transporters (Grinberg et al., 2020). There are also studies that show the fly model may be relevant for studying the development of neurodegenerative diseases (AD, PD) and the potential for their probiotic treatment (Kong et al., 2018; Westfall et al., 2019). In a drosophila AD disease model, enterobacteria infection aggravated the progression of AD by increasing the infiltration of immune hemocyte to the brain and thus exacerbating TNF-JNK mediated neurodegeneration (Wu et al., 2017). Administration of the polyphenol eigallocatechin-3-gallate in a PD drosophila model rescued dopaminergic, survival, and behavioral deficits, with profound changes in gut microbiota composition and restoring the abundance of a set of bacteria (Xu et al., 2020). Notably, the protection was blunted when gut microbiota was disrupted by antibiotics.

Despite these microbiota-mediated behavioral effects observed in flies, there have been inconsistencies reported. For example, no significant increase in the activity of axenic flies relative to those with normal microbiome has been shown (Selkrig *et al.*, 2018), as opposed to the hyperactivity in axenic flies previously reported (Schretter *et al.*, 2018). Furthermore, microbiota-led changes in courtship were not observed in some (Leftwich et al., 2017; Selkrig et al., 2018), while they were reported in other studies (Qiao et al., 2019; Sharon et al., 2010). These variable results may stem from changes in experimental design and environmental parameters. Some of

the factors that can contribute to the varied results in microbiome studies in drosophila are host age, nutrient variability, species and strains of microbiota, and female mating status (Delbare et al., 2020; Staubach et al., 2013; Wong et al., 2013, 2015).

The complex interplay between the microbiota and dietary nutrition has also been studied in flies (Grenier and Leulier, 2020; Keebaugh et al., 2018; Wong et al., 2014). Important insights that have emerged from this body of literature focus on how microbiota can shape behavioral decisions and life history traits. Using a chemically defined diet, the commensal bacteria *Acetobacter pomorum* and *Lactobacilli*, together but not alone, were shown to rescue the behavioural and reproductive effects caused by the lack of dietary essential amino acids (Leitão-Gonçalves *et al.*, 2017). These commensals engage in metabolic cross-feeding to become resilient to detrimental host diets. Using isotope-resolved metabolomics, a syntrophic interplay between *Acetobacter pomorum* and *Lactobacillus plantarum* could be demonstrated, wherein lactate generated by the lactobacillus is used up by the acetobacter to generate amino acids that are essential for lactobacillus to grow in imbalance diets (Henriques *et al.*, 2020). Remarkably, lactate was found to be necessary and sufficient for acetobacter to alter the fly's protein appetite and feeding decision-making behaviour. In a recent study, gut enterocytes were shown to sense the levels of diet- and microbiome-derived essential amino acids and relay signal to brain through the neuropeptide CNMamide to trigger compensatory appetite for essential amino acids (Kim et al., 2021). In another example of microbe-microbe interaction and its influence on fly behavior, Saccharomyces-Acetobacter co-culture was shown to regulate Drosophila olfactory and egg-laying behaviors distinctly than individual components (Fischer *et al.*, 2017). Saccharomyces-derived ethanol was metabolized by the Acetobacter to acetate, which along with its metabolic derivatives were essential for co-culture preference and altering the egg-laying behaviour. In other reports on life-history traits, the gut microbiota has been presented as a key player in development and olfactory-guided foraging behaviours (Qiao et al., 2019; Wong et al., 2017). In a foraging assay, conventional flies preferred the associated bacterial strains *L. plantarum* and *A. pomorum*, suggesting the role of microbiota in the microbial preference of flies. In case of axenic flies, the preference for *Lactobacillus* was retained while for *Acetobacter* was abolished.These microbial preferences were shown to be olfactory guided, and have large influence on host foraging, which is also regulated by dietary nutrients (Wong et al., 2017). Taken together, these studies provide a solid framework wherein questions such as how hosts become populated with specific community of microbes in their natural environment, can be thoroughly investigated.

The nematode worm (*Caenorhabditis elegans*): Utility as a model organism for microbiota – brain communication.

With a complete neural connectome and developmental cell lineage mapped as well as plethora of genetic resources and rich behavioral repertoire, *C. elegans* has been influential in outlining conserved genetic pathways and dissection of behaviour at the level of genes, individual neurons, and neural circuits (Corsi et al., 2015). The breadth of behaviours include swimming and crawling, responses to a wide variety of sensory cues that are modulated by internal states, egg-laying, mating and social feeding, along with habituation and associative learning. Coupled to inherent

strengths such as a short life cycle and lifespan, transparent body, a large isogenic brood size and an exceptional ease to be made germ-free, *C. elegans* is emerging as a strong high-throughput system for undertstanding host-microbiome interactions (Zhang et al., 2017a). Although, typically *C. elegans* are cultivated in the laboratory on *Escherichia coli* OP50 seeded on agar plates, their natural habitat of rotting plant matter is a microbe-rich environment where the bacteria can be source of nutrition or can be pathogenic and a source of infection (Schulenburg and Félix, 2017). Since bacteria are both diet and microbiota, not all bacterial strains can colonize the animal. The microbiota analysis of the natural environment of wild *C. elegans* has identified a community comprising of *Proteobacteria, Bacteroidetes, Firmicutes*, and *Actinobacteria* (Samuel *et al.*, 2016), specifically enriched in *alpha-Proteobacteria* genera such as *Ochrobactrum* and *Pseudomonas* which colonize the intestine (Dirksen *et al.*, 2016). Recently, a specific model microbiome for use in C. elegans (CeMbio) has been reported, which comprises of 12 strains of bacteria from 9 distinct families that are routinely present in natural *C. elegans*, irrespective of origin and isolating laboratory (Dirksen *et al.*, 2020). The development of this simplified core microbiome along with other available bacterial mutant libraries from targeted deletion or transposon insertion collections (Govindan et al., 2015; Watson et al., 2014) are valuable resources that complement the highly tractable *C. elegans* host, which is amenable to genome-wide reverse genetic screens using RNAi libraries (Xiao *et al.*, 2015). This will further advance a mechanistic understanding of the microbiome impact on host health and disease. Next, we focus our attention on some of the recent studies reporting effects of microbiota on the nervous system and behaviour in *C. elegans*, particularly the role of commensal and probiotic bacteria.

Effects of microbiota manipulation on brain and behaviour.

C. elegans innately recognize nutritive as well as pathogenic bacteria in its environment using chemical and mechanical cues and generate rapid behavioural responses (Meisel and Kim, 2014). Worms exhibit robust chemotactic responses towards organic volatile molecules of bacterial origin such as diacetyl and isoamylalcohol, which have been found to be released by bacterial food in the natural habitat of *C. elegans* (Choi et al., 2016b; Worthy et al., 2018). Among these attractive bacterial isolates, *Providencia sp.* JUb39 and its role in modulating host sensory behavior has been the subject of a recent impressive study aiming at understanding the mechanistic basis of this microbiota–brain signalling (O'Donnell *et al.*, 2020). Here, the authors showed that the neuromodulator tyramine (a biogenic amine) produced by the commensal *Providencia* bacteria, which colonize the gut, is converted to octopamine (norepinephrine equivalent in worms) by the *C. elegans* host and manipulates aversive sensory responses of the host. Using a *C. elegans* mutant of the *cat-1* gene, a vesicular monoamine transporter, the authors further demonstrated that the neuronal transport of octopamine was essential for the change in behaviour driven by the bacteria. Interestingly, such an intervention in worms has been considered to be the functional equivalent to the vagotomy (surgical disruption of the vagus nerve) in mice (Matty and Chalasani, 2020), that has been shown to disrupt microbiota-induced behavioural alterations (Bravo *et al.*, 2011). Furthermore, *C. elegans* colonized by *Providencia* exhibit a selective bias for these bacteria in food choice assays, which is dependent on tyramine produced by bacteria and host octopamine signalling (O'Donnell et al., 2020). These results demonstrate that the biogenic amine neurotransmitter produced by the gut bacteria can override

the host control of a sensory decision by mimicking the function of the cognate host molecule, and thereby promote both host and microbe fitness. Recent studies indicate that human gut microbiota also harbour many member species closely related to *Providencia*, that can modulate biogenic amines (Pugin *et al.*, 2017), thereby hinting at the commonality of the microbial factors altering host physiology across the animal phyla. In another impressive study on gut-brain interaction in modulating behaviour in worms, an enteric serotonergic neuron was found to acutely detect food ingestion via DEL-3 and DEL-7, ion channels from the evolutionarily conserved ASIC family (Rhoades *et al.*, 2019).These channels localize to the sensory endings of the enteric neuron in the gut and are required for the feeding-dependent neuronal activation, which leads to slowing of the locomotion while the animal feeds. Furthermore, the neuronal activation appears to be mediated by a bacterial component that is heat stable, linking microbial recognition in the gut to the alteration of the feeding behavior.

Apart from the chemotaxis towards volatile cues, C. elegans also exhibit robust detection and response to gaseous cues of oxygen and carbon dioxide that are a result of bacterial metabolism. The gas-sensing signalling molecules, neural circuits and natural genetic variations have been extensively studied in *C. elegans* over the past 2 decades (Carrillo and Hallem, 2015). In the absence of bacterial food, worms exhibit a preference for ~8% oxygen levels in their environment (ambient laboratory oxygen levels are ~21%) (Gray *et al.*, 2004). This hyperoxia avoidance shown by the wild-type strain, N2, is greatly reduced in the presence of bacteria. This altered aerotaxis behaviour in the presence of bacteria is regulated by allelic variation in the *npr-1* gene, encoding a GPCR homologous to the mammalian neuropeptide Y receptor (de Bono and Bargmann, 1998). NPR-1 dependent signaling circuitry, induced by the presence of bacteria, also has a profound influence on various other behavioural phenotypes, including 'social feeding' or aggregation in feeding behaviour, as well as roaming versus dwelling locomotion decisions (Cheung et al., 2005; Gray et al., 2004; Macosko et al., 2009). Interestingly, NPR-1 dependent behaviors in N2 worms were found to be abolished in the presence of exopolysaccharide matrix producing mucoid strains of bacteria, suggesting a potential mechanism through which biofilm synthesis by a bacterial population may impede specific host responses to microbes (Reddy *et al.*, 2011).

In the domain of studies linking microbiome and neurodegenerative diseases, the mechanisms as to how bacterial metabolites directly modulate the neuronal degeneration are largely underexplored. In a recent study, the authors used a model of progressive degeneration of touch receptor neurons in *C. elegans* as a surrogate of neurodegeneration and evaluated the neurodegeneration as a function of of administration of various dietary bacteria (Urrutia *et al.*, 2020). The bacteria *E. coli* HT115*, E. coli* K-12*, C. aquatica, P. aeruginosa, S. humi*, and *B. pumilus* promoted neuroprotection, with *E.coli* HT115 significantly protected mechanoreceptors and interneurons of the touch receptor circuit on a long-term basis. This was mediated through the bacterial GABA and host insulin/IGF-1-like signaling pathway. The involvement of bacterial GABA in neuroprotection in worms is interesting, in light of the observations that the bacterial GABA is one of the main effectors of microbiota on the human CNS and the relative abundance of GABA producing gut bacteria negatively correlates with depression-associated symptoms in patients (Strandwitz *et al.*, 2019; Valles-Colomer *et al.*, 2019). Moving forward, the answers to the questions of how GABA and other microbiota-produced neuroactive molecules influence the processes in brain and other distal tissues, will be keenly sought. In another recent study

highlighting the microbial modulation of neurodegeneration in worms, the authors used a *C. elegans* model of synucleinopathy with ectopic expression of human alpha-synuclein (AS) and observed that administration of the probiotic bacterium *Bacillus subtilis* PXN21 to *C. elegans* inhibited, delayed, and reversed AS aggregation (Goya et al., 2020). The neuroprotection conferred by *B. subtilis* during aging late in life is mediated by the formation of biofilm in the gut and its metabolites, nitric oxide (NO) and quorum-sensing pentapeptide competence sporulation stimulating factor (CSF). However, in young adults, the bacteria was found to confer neuroprotection partly through metabolites distinct than NO and independent of gut colonization. Furthermore, the authors demonstrated that the probiotic inhibited α-syn aggregation by promoting the activation of host protective pathways, including DAF-16/FOXO and sphingolipid metabolism. These findings prompt further investigations into the protective role of the probiotic *B. subtilis* and its potential use as a diet-based intervention for Parkinson's disease. We are also witnessing a meteoric rise in the number of studies highlighting the communication between the microbiota, nervous and immune systems in health and disease (Fung et al., 2017) and *C. elegans* as a model is contributing immensely to a greater understanding of microbiota's role at the neuro-immune interface (Singh and Aballay, 2020).

Moving Forward with Model Organisms: Focus on Social and Stress-related Behaviours

French poet and novelist, Victor Hugo, once remarked, "Nothing else in the world is so powerful as an idea whose time has come". Today, the role of microbiota in health and disease, particularly in the context of brain and behavior, appears to be one such idea. To tilt the balance of hope vs hype towards hope, particularly in the context of microbiome and mental health, we need rigorous studies to go beyond disease associations and to translate this knowledge into therapeutics (Taylor, 2019). The two major behavioral domains, where the microbiota is shown to have the most robust and remarkable effects, are in the modulation of social behavior and organismal stress response. We envisage a tangible translational progress in the model-system led understanding of microbiota-brain interactions in the fields of social and stress behaviour, which are summarized below.

Stress and social behaviors are intricately intertwined: exposure to traumatic stress profoundly impacts social interactions, and conversely, social environment is a strong predictor of our ability to cope with stress (Hostinar et al., 2014; Sandi and Haller, 2015). Moreover, both response systems exhibit a great degree of cross-talk in the neuromodulatory and neuroanatomical substrates, which could be amenable to microbial modulation during development and acutely. A recent study described how the microbiome can modulate social behaviours through neural circuits underlying stress responses in the rodent brain (Wu et al., 2021). Using GF and antibiotic-treated mice, the authors showed that gut microbiota affects neural activity in PVN of hypothalamus of male mice to regulate typical stress responses such as corticosterone levels as well as social behaviours (Wu et al., 2021). A bacterial species, *Enterococcus faecalis*, was identified to promote social behaviour and reduce corticosterone levels in mice upon social stress.

Social Behavior and Microbiome

Enormous advances in organismal biology over many decades have firmly established genetics, development and environment as the key determinants shaping social behavior across the animal kingdom. A new appreciation of host-microbe interactions has led to the emergence of the study of sociability from a microbiota point of view (Sherwin *et al.*, 2019). Germ-free rodents display remarkably different social behaviour as compared to ones colonized with bacteria and probiotic supplementation can reverse social deficits in early life as well as adulthood. This suggests that microbial signals play an important role in normal social neurodevelopment and programming in the brain. Understanding causal mechanisms underlying this intriguing association could potentially lead to new therapies for social disorders in humans, such as ASD, social anxiety disorder and the social deficits associated with schizophrenia.

Simple model organisms such as *C. elegans*, Drosophila, and zebrafish exhibit a variety of social behaviors, including aggregation, courtship and mating, aggression, and shoaling (Geng and Peterson, 2019; Sokolowski, 2010), similar to those in more complex animals. The unsurpassable access to the genes-neurons-circuits, and the precise control over the environment of the social context (Robinson *et al.*, 2019) along with the low-dimensional microbiota in these organisms, provides a tremendous opportunity to seek causal mechanisms linking social behavior and microbiome (**Figure 3**). Oxytocinergic signaling, that is conserved from worms to humans (Grinevich and Neumann, 2020; Odekunle and Elphick, 2020), and the corresponding neural circuits may be the key convergent node between the microbial cues and the higher order brain functioning and the emergent social behaviour (Erdman, 2021). There is further supported by the observation that probiotic *L. reuteri,* as mentioned previously*,* was able to rescue social interaction-induced synaptic plasticity of ASD mice, but not in mice lacking the oxytocin receptor in dopaminergic neurons (Sgritta *et al.*, 2019).

Stress and Microbiome

Allostatic load of stress remains one of the most potent risk factors for psychiatric illness (Juster et al., 2010). There is a robust bi-directional communication between the stress response system and state of the microbiota: a number of studies have demonstrated that stress alters the gut microbiota composition across a variety of species, and conversely the gut microbiota also shapes the stress responsivity, primarily via the modulation of the hypothalamus-pituitary-adrenal (HPA) axis (Foster et al., 2017; de Weerth, 2017). Moreover, specific bacterial strains have been shown to reverse various stress effects in several animal models (Long-Smith et al., 2020). With a plethora of association and correlation studies laying the groundwork, the field of stress-microbiota communication is ripe for causative analysis (**Figure 4**). The simpler model organisms and their neurogenetic accessibility could be leveraged to obtain this mechanistic analysis. While the invertebrates (*C. elegans* and Drosophila) have comparable neuropeptidergic modulatory systems and simpler microbiome (Jékely et al., 2018; Liu et al., 2018; Masuzzo et al., 2020), the use of zebrafish, with robust behavioral paradigms and strong similarities in stress-related neuroanatomy and hormones (Biran et al., 2018; Stewart et al., 2015), may be a game-changer for the field. Notably, zebrafish and humans share structural and functional homology in the HPA axis (Nagpal *et al.*, 2019), of which the PVN of the hypothalamus is of particular relevance for

stress regulation and microbiome modulation (Farzi et al., 2018). Moreover, hypothalamo-neurohypophyseal system, lacking a tight blood-brain barrier (Anbalagan et al., 2018), is a likely target to be impacted by the microbial metabolites during development which in zebrafish being external, can be harnessed for easy manipulation of microbiota environment and microscopic visualization of developing circuits. The mechanisms and lessons learnt from such studies would greatly complement and guide the research in mammalian systems. This cross-species synergy is crucial and paramount for the development of next generation microbiota-based therapeutic options (psychobiotics) for stress-related conditions including anxiety, depression and post-traumatic stress disorder (Dinan et al., 2013).

Promises and Caveats

C. elegans, Drosophila, and Zebrafish, all display robust stress and social responses employing molecules and circuits similar to those in mice and humans, which are amenable to microbial modulation. A cross-species approach presents a golden opportunity to realise mechanistic dissection of microbiota-brain interactions in the context of these behaviors, which have far-reaching implications for human therapeutics for neuropsychiatric and neurodevelopmental disorders. Furthermore, as has been demonstrated before for life-history traits, these models are tailor-made to conduct high-throughput screens to define the underlying host-microbe-drug-nutrient interactions (Pryor et al., 2019; Scott et al., 2017; Wang et al., 2015). Thus, it can be foreseen that, in the light of the scalability offered by these simple models, it will be possible to screen microbes and microbial metabolites relevant for the diseases of the nervous system. One, of course, needs to consider and understand the limitations associated with these systems and an overall simplification may compromise, in some cases, the physiological relevance. For instance, the gut microbiota composition of these simpler organisms differs dramatically to that of the mammals, and more so the bacteria are mostly aerobic, unlike the obligate anaerobic bacteria dominating the human gut landscape. There are some species-specific limitations as well. Zebrafish poses logistical issues in terms of time, cost, and means to raise GF larvae to adulthood, thereby restricting the mechanistic studies to early life-stages. The methods to genetically transform some microbial partners, in the case of Drosophila, are not well developed and microbiome experiments with flies and worms need to be carefully designed and interpreted, because they are microbivores. *C. elegans* do not possess a well-defined blood-brain barrier, an important anatomical feature in vertebrate brains which regulates the access of microbially derived metabolites. Thus, signals generated in the gut of the worms are more likely to have systemic effects in *C. elegans* than in mammals. Despite the limitations, the experimental systems of worms, flies and fish, by virtue of yielding fine-grained mechanistic results faster and more economically, are a perfect complement to the mouse and human studies (Douglas, 2018).

Outlook

To realize the true potential of the microbiota-brain research, the two major issues that need to be addressed, going forward, are mechanistic causal rigor and human translatability. Though, ‘human microbiota-associated animals’ (Arrieta et al., 2016) – where FMT from human subjects

into microbiota-depleted animals is followed by a phenotypic analysis in the recipient animals, has been the benchmark for causality, the majority positive studies suffer from unsuitable statistical analyses, improper experimental designs, and bias (Walter et al., 2020), generating an unrealistic expectation. For the long-term reliability of microbiomebrain research and to facilitate its translation, causal candidate elements of the gut microbiota (specific bacterial taxa, metabolites, etc.) should be identified following an unbiased hypothesis-generating integrative multi-omic analysis with host nervous system phenotypes, which subsequently be confirmed with functional experiments in gnotobiotic animals or disease models performed without pooling donor samples and statistical pseudo replication (Nyholm et al., 2020; Walter et al., 2020). Moreover, novel statistical and machine learning approaches, could be adopted to microbiome data and be used to infer causality and mechanistic interactions directly in humans, where longitudinal studies and randomized controlled trials are the need of the hour.

As Yeats alluded to almost a century ago, essence of the chestnut tree is neither to be found in the leaf, nor the blossom, nor the trunk; essence of the ‘great rooted blossomer’ lies in the tree as a whole. Understanding of the host physiology and in particular, nervous system structure-function and the emergent behavior, should increasingly take into account the whole ‘holobiont’ (Nagpal and Cryan, 2021; Theis et al., 2016). Although majority of efforts over the past two decades have investigated the microbiota-gut-brain axis in rodents, it’s time we dig into mechanisms leveraging a cross-species approach and make robust and large human translational studies a central focus.

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Declaration of Interests

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Figure and Table Legends

***Figure 1**: Microbiota-gut-brain signaling in humans and in model systems of Caenorhabditis elegans, Drosophila melanogaster, zebrafish, and mouse.*

***Figure 2**: The gut microbiota associated with different model species and the variety of microbial metabolites relaying the communication between the microbiota and the brain. Microbially produced metabolites in both pathogenic and commensal contexts have been demonstrated to modulate brain function, behaviour and life-history traits.*

***Figure 3**. Microbiome and social behavior. The state of microbiota and its association with sociability should be tested for causality using direct administration of putative microbial 'sociobiotics' and ethologically relevant behaviours.*

***Figure 4**: Bidirectional communication between stress and microbiome. Multiple species-specific stressors will be key to understand this dynamic interplay between the stress response pathways and circuits, and the alterations in the state of microbiota.*

***Table 1**: Selected Examples of Microbiota-Gut-Brain axis interactions in Neurobehavioural Disorders in Preclinical Rodent Models.*

References

1. Abildgaard, A., Kern, T., Pedersen, O., Hansen, T., Lund, S., and Wegener, G. (2021). A diet-induced gut microbiota component and related plasma metabolites are associated with depressive-like behaviour in rats. Eur. Neuropsychopharmacol. *43*, 10–21.
2. Anbalagan, S., Gordon, L., Blechman, J., Matsuoka, R.L., Rajamannar, P., Wircer, E., Biran, J., Reuveny, A., Leshkowitz, D., Stainier, D.Y.R., et al. (2018). Pituicyte Cues Regulate the Development of Permeable Neuro-Vascular Interfaces. Dev. Cell 1–16.
3. Ansaldo, E., Farley, T.K., and Belkaid, Y. (2021). Control of immunity by the Microbiota. Annu. Rev. Immunol. *39*, 449–479.
4. Arias-Jayo, N., Alonso-Saez, L., Ramirez-Garcia, A., and Pardo, M.A. (2018). Zebrafish Axenic Larvae Colonization with Human Intestinal Microbiota. Zebrafish *15*, 96–106.
5. Arrieta, M.-C., Walter, J., and Finlay, B.B. (2016). Human Microbiota-Associated Mice: A Model with Challenges. Cell Host Microbe *19*, 575–578.
6. Bailey, M.T., Dowd, S.E., Galley, J.D., Hufnagle, A.R., Allen, R.G., and Lyte, M. (2011). Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. Brain Behav. Immun. *25*, 397–407.
7. Bastiaanssen, T.F.S., Gururajan, A., van de Wouw, M., Moloney, G.M., Ritz, N.L., Long-Smith, C.M., Wiley, N.C., Murphy, A.B., Lyte, J.M., Fouhy, F., et al. (2021). Volatility as a concept to understand the impact of stress on the microbiome. Psychoneuroendocrinology *124*, 105047.
8. Bercik, P., Park, A.J., Sinclair, D., Khoshdel, A., Lu, J., Huang, X., Deng, Y., Blennerhassett, P.A., Fahnestock, M., Moine, D., et al. (2011a). The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut-brain communication. Neurogastroenterol. Motil. *23*, 1132–1139.
9. Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., Deng, Y., Blennerhassett, P., Macri, J., McCoy, K.D., et al. (2011b). The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology *141*, 599–609, 609.e1-3.
10. Berer, K., Mues, M., Koutrolos, M., Rasbi, Z.A., Boziki, M., Johner, C., Wekerle, H., and Krishnamoorthy, G. (2011). Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. Nature *479*, 538–541.
11. Berer, K., Gerdes, L.A., Cekanaviciute, E., Jia, X., Xiao, L., Xia, Z., Liu, C., Klotz, L., Stauffer, U., Baranzini, S.E., et al. (2017). Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. Proc. Natl. Acad. Sci. U. S. A. *114*, 10719–10724.
12. 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., et al. (2021). The microbial metabolite p-Cresol induces autistic-like behaviors in mice by remodeling the gut microbiota. Microbiome *9*, 157.
13. Berthoud, H.-R. (2008). Vagal and hormonal gut-brain communication: from satiation to satisfaction. Neurogastroenterol. Motil. *20 Suppl 1*, 64–72.
14. Bharwani, A., West, C., Champagne-Jorgensen, K., McVey Neufeld, K.-A., Ruberto, J., Kunze, W.A., Bienenstock, J., and Forsythe, P. (2020). The vagus nerve is necessary for the rapid and widespread neuronal activation in the brain following oral administration of psychoactive bacteria. Neuropharmacology *170*, 108067.
15. Biran, J., Blechman, J., Wircer, E., and Levkowitz, G. (2018). Development and Function of the Zebrafish Neuroendocrine System. Model Animals in Neuroendocrinology 101–131.
16. Blacher, E., Bashiardes, S., Shapiro, H., Rothschild, D., Mor, U., Dori-Bachash, M., Kleimeyer, C., Moresi, C., Harnik, Y., Zur, M., et al. (2019). Potential roles of gut microbiome and metabolites in modulating ALS in mice. Nature *572*, 474–480.
17. de Bono, M., and Bargmann, C.I. (1998). Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in C. elegans. Cell *94*, 679–689.
18. Borrelli, L., Aceto, S., Agnisola, C., De Paolo, S., Dipineto, L., Stilling, R.M., Dinan, T.G., Cryan, J.F., Menna, L.F., and Fioretti, A. (2016). Probiotic modulation of the microbiota-gut-brain axis and behaviour in zebrafish. Sci. Rep. *6*, 30046.
19. Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., Bienenstock, J., and Cryan, J.F. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc. Natl. Acad. Sci. U. S. A. *108*, 16050–16055.
20. Brembs, B. (2019). Genetic Analysis of Behavior in Drosophila. In The Oxford Handbook of Invertebrate

Neurobiology, J.H. Byrne, ed. (Oxford University Press). https://doi.org/10.1093/oxfordhb/9780190456757.013.37.
21. Brown, A.J., Goldsworthy, S.M., Barnes, A.A., Eilert, M.M., Tcheang, L., Daniels, D., Muir, A.I., Wigglesworth, M.J., Kinghorn, I., Fraser, N.J., et al. (2003). The Orphan G Protein-coupled Receptors GPR41 and GPR43 Are Activated by Propionate and Other Short Chain Carboxylic Acids *. J. Biol. Chem. *278*, 11312–11319.
22. Bruckner, J.J., Stednitz, S.J., Grice, M.Z., and Larsch, J.J. (2020). The microbiota promotes social behavior by neuro-immune modulation of neurite complexity. BioRxiv. https://doi.org/10.1101/2020.05.01.071373.
23. Buchon, N., Silverman, N., and Cherry, S. (2014). Immunity in Drosophila melanogaster--from microbial recognition to whole-organism physiology. Nat. Rev. Immunol. *14*, 796–810.
24. Buffington, S.A., Di Prisco, G.V., Auchtung, T.A., Ajami, N.J., Petrosino, J.F., and Costa-Mattioli, M. (2016). Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. Cell *165*, 1762–1775.
25. Buffington, S.A., Dooling, S.W., Sgritta, M., Noecker, C., Murillo, O.D., Felice, D.F., Turnbaugh, P.J., and Costa-Mattioli, M. (2021). Dissecting the contribution of host genetics and the microbiome in complex behaviors. Cell *84*, 1740-1756.e16.
26. Burberry, A., Wells, M.F., Limone, F., Couto, A., Smith, K.S., Keaney, J., Gillet, G., van Gastel, N., Wang, J.-Y., Pietilainen, O., et al. (2020). C9orf72 suppresses systemic and neural inflammation induced by gut bacteria. Nature *582*, 89–94.
27. Burne, T., Scott, E., van Swinderen, B., Hilliard, M., Reinhard, J., Claudianos, C., Eyles, D., and McGrath, J. (2011). Big ideas for small brains: what can psychiatry learn from worms, flies, bees and fish? Mol. Psychiatry *16*, 7–16.
28. Carrillo, M.A., and Hallem, E.A. (2015). Gas sensing in nematodes. Mol. Neurobiol. *51*, 919–931.
29. Catron, T.R., Swank, A., Wehmas, L.C., Phelps, D., Keely, S.P., Brinkman, N.E., McCord, J., Singh, R., Sobus, J., Wood, C.E., et al. (2019). Microbiota alter metabolism and mediate neurodevelopmental toxicity of 17β-estradiol. Sci. Rep. *9*, 7064.
30. Cekanaviciute, E., Yoo, B.B., Runia, T.F., Debelius, J.W., Singh, S., Nelson, C.A., Kanner, R., Bencosme, Y., Lee, Y.K., Hauser, S.L., et al. (2017). Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. Proc. Natl. Acad. Sci. U. S. A. *114*, 10713–10718.
31. Chandler, J.A., Lang, J.M., Bhatnagar, S., Eisen, J.A., and Kopp, A. (2011). Bacterial communities of diverse Drosophila species: ecological context of a host-microbe model system. PLoS Genet. *7*, e1002272.
32. Chaston, J.M., Newell, P.D., and Douglas, A.E. (2014). Metagenome-wide association of microbial determinants of host phenotype in Drosophila melanogaster. MBio *5*, e01631-14.
33. Chen, H., Nwe, P.-K., Yang, Y., Rosen, C.E., Bielecka, A.A., Kuchroo, M., Cline, G.W., Kruse, A.C., Ring, A.M., Crawford, J.M., et al. (2019a). A Forward Chemical Genetic Screen Reveals Gut Microbiota Metabolites That Modulate Host Physiology. Cell *177*, 1217-1231.e18.
34. Chen, K., Luan, X., Liu, Q., Wang, J., Chang, X., Snijders, A.M., Mao, J.H., Secombe, J., Dan, Z., Chen, J.H., et al. (2019b). Drosophila Histone Demethylase KDM5 Regulates Social Behavior through Immune Control and Gut Microbiota Maintenance. Cell Host Microbe *25*, 537-552.e8.
35. Chen, R.Y., Mostafa, I., Hibberd, M.C., Das, S., Mahfuz, M., Naila, N.N., Islam, M.M., Huq, S., Alam, M.A., Zaman, M.U., et al. (2021). A Microbiota-Directed Food Intervention for Undernourished Children. N. Engl. J. Med. *384*, 1517–1528.
36. Chen, S.G., Stribinskis, V., Rane, M.J., Demuth, D.R., Gozal, E., Roberts, A.M., Jagadapillai, R., Liu, R., Choe, K., Shivakumar, B., et al. (2016). Exposure to the Functional Bacterial Amyloid Protein Curli Enhances Alpha-Synuclein Aggregation in Aged Fischer 344 Rats and Caenorhabditis elegans. Sci. Rep. *6*, 34477.
37. Cheung, B.H.H., Cohen, M., Rogers, C., Albayram, O., and de Bono, M. (2005). Experience-dependent modulation of C. elegans behavior by ambient oxygen. Curr. Biol. *15*, 905–917.
38. Chia, J.S.M., Wall, E.S., Wee, C.L., Rowland, T.A.J., Cheng, R.-K., Cheow, K., Guillemin, K., and Jesuthasan, S. (2019). Bacteria evoke alarm behaviour in zebrafish. Nat. Commun. *10*, 3831.
39. Choi, G.B., Yim, Y.S., Wong, H., Kim, S., Kim, H., Kim, S.V., Hoeffer, C.A., Littman, D.R., and Huh, J.R. (2016a). The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. Science *351*, 933–939.
40. Choi, J.I., Yoon, K.-H., Subbammal Kalichamy, S., Yoon, S.-S., and Il Lee, J. (2016b). A natural odor attraction between lactic acid bacteria and the nematode Caenorhabditis elegans. ISME J. *10*, 558–567.
41. Cholan, P.M., Han, A., Woodie, B.R., Watchon, M., Kurz, A.R.M., Laird, A.S., Britton, W.J., Ye, L., Holmes, Z.C., McCann, J.R., et al. (2020). Conserved anti-inflammatory effects and sensing of butyrate in zebrafish. Gut Microbes *12*, 1–11.
42. Citraro, R., Lembo, F., De Caro, C., Tallarico, M., Coretti, L., Iannone, L.F., Leo, A., Palumbo, D., Cuomo, M.,

Buommino, E., et al. (2021). First evidence of altered microbiota and intestinal damage and their link to absence epilepsy in a genetic animal model, the WAG/Rij rat. Epilepsia *62*, 529–541.
43. Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R.D., Shanahan, F., Dinan, T.G., and Cryan, J.F. (2013). The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. Mol. Psychiatry *18*, 666–673.
44. Cohen, L.J., Esterhazy, D., Kim, S.-H., Lemetre, C., Aguilar, R.R., Gordon, E.A., Pickard, A.J., Cross, J.R., Emiliano, A.B., Han, S.M., et al. (2017). Commensal bacteria make GPCR ligands that mimic human signalling molecules. Nature *549*, 48–53.
45. Collins, S.M., Surette, M., and Bercik, P. (2012). The interplay between the intestinal microbiota and the brain. Nat. Rev. Microbiol. *10*, 735–742.
46. Colosimo, D.A., Kohn, J.A., Luo, P.M., Piscotta, F.J., Han, S.M., Pickard, A.J., Rao, A., Cross, J.R., Cohen, L.J., and Brady, S.F. (2019). Mapping Interactions of Microbial Metabolites with Human G-Protein-Coupled Receptors. Cell Host Microbe *26*, 273-282.e7.
47. Coretti, L., Cristiano, C., Florio, E., Scala, G., Lama, A., Keller, S., Cuomo, M., Russo, R., Pero, R., Paciello, O., et al. (2017). Sex-related alterations of gut microbiota composition in the BTBR mouse model of autism spectrum disorder. Sci. Rep. *7*, 45356.
48. Corsi, A.K., Wightman, B., and Chalfie, M. (2015). A Transparent Window into Biology: A Primer on Caenorhabditis elegans. Genetics *200*, 387–407.
49. Cowan, C.S.M., Dinan, T.G., and Cryan, J.F. (2020). Annual Research Review: Critical windows - the microbiota-gut-brain axis in neurocognitive development. J. Child Psychol. Psychiatry *61*, 353–371.
50. Cox, L.M., Maghzi, A.H., Liu, S., Tankou, S.K., Dhang, F.H., Willocq, V., Song, A., Wasén, C., Tauhid, S., Chu, R., et al. (2021). Gut Microbiome in Progressive Multiple Sclerosis. Ann. Neurol. *89*, 1195–1211.
51. Cruz-Pereira, J.S., Rea, K., Nolan, Y.M., O'Leary, O.F., Dinan, T.G., and Cryan, J.F. (2020). Depression's Unholy Trinity: Dysregulated Stress, Immunity, and the Microbiome. Annu. Rev. Psychol. *71*, 49–78.
52. Cryan, J.F., and Dinan, T.G. (2012). Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. Nat. Rev. Neurosci. *13*, 701–712.
53. Cryan, J.F., O'riordan, K.J., Cowan, C.S.M., Sandhu, K.V., Bastiaanssen, T.F.S., Boehme, M., Codagnone, M.G., Cussotto, S., Fulling, C., Golubeva, A.V., et al. (2019). The microbiota-gut-brain axis. Physiol. Rev. *99*, 1877–2013.
54. Cryan, J.F., O'Riordan, K.J., Sandhu, K., Peterson, V., and Dinan, T.G. (2020). The gut microbiome in neurological disorders. Lancet Neurol. *19*, 179–194.
55. Cuomo, M., Borrelli, L., Della Monica, R., Coretti, L., De Riso, G., D'Angelo Lancellotti di Durazzo, L., Fioretti, A., Lembo, F., Dinan, T.G., Cryan, J.F., et al. (2021). DNA Methylation Profiles of Tph1A and BDNF in Gut and Brain of L. Rhamnosus-Treated Zebrafish. Biomolecules *11*.
56. Davis, D.J., Bryda, E.C., Gillespie, C.H., and Ericsson, A.C. (2016a). Microbial modulation of behavior and stress responses in zebrafish larvae. Behav. Brain Res. *311*, 219–227.
57. Davis, D.J., Doerr, H.M., Grzelak, A.K., Busi, S.B., Jasarevic, E., Ericsson, A.C., and Bryda, E.C. (2016b). Lactobacillus plantarum attenuates anxiety-related behavior and protects against stress-induced dysbiosis in adult zebrafish. Sci. Rep. *6*, 1–11.
58. De Palma, G., Lynch, M.D.J., Lu, J., Dang, V.T., Deng, Y., Jury, J., Umeh, G., Miranda, P.M., Pigrau Pastor, M., Sidani, S., et al. (2017). Transplantation of fecal microbiota from patients with irritable bowel syndrome alters gut function and behavior in recipient mice. Sci. Transl. Med. *9*.
59. Delbare, S.Y.N., Ahmed-Braimah, Y.H., Wolfner, M.F., and Clark, A.G. (2020). Interactions between the microbiome and mating influence the female's transcriptional profile in Drosophila melanogaster. Sci. Rep. *10*, 18168.
60. Depetris-Chauvin, A., Galagovsky, D., Chevalier, C., Maniere, G., and Grosjean, Y. (2017). Olfactory detection of a bacterial short-chain fatty acid acts as an orexigenic signal in Drosophila melanogaster larvae. Sci. Rep. *7*, 14230.
61. Desbonnet, L., Clarke, G., Shanahan, F., Dinan, T.G., and Cryan, J.F. (2014). Microbiota is essential for social development in the mouse. Mol. Psychiatry *19*, 146–148.
62. Diaz Heijtz, R., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., Hibberd, M.L., Forssberg, H., and Pettersson, S. (2011). Normal gut microbiota modulates brain development and behavior. Proc. Natl. Acad. Sci. U. S. A. *108*, 3047–3052.
63. Dinan, T.G., Stanton, C., and Cryan, J.F. (2013). Psychobiotics: a novel class of psychotropic. Biol. Psychiatry *74*, 720–726.
64. Dirksen, P., Marsh, S.A., Braker, I., Heitland, N., Wagner, S., Nakad, R., Mader, S., Petersen, C., Kowallik, V., Rosenstiel, P., et al. (2016). The native microbiome of the nematode Caenorhabditis elegans: gateway to a new

host-microbiome model. BMC Biol. *14*, 38.
65. Dirksen, P., Assié, A., Zimmermann, J., Zhang, F., Tietje, A.-M., Marsh, S.A., Félix, M.-A., Shapira, M., Kaleta, C., Schulenburg, H., et al. (2020). CeMbio - The Caenorhabditis elegans Microbiome Resource. G3 *10*, 3025–3039.
66. Dodiya, H.B., Kuntz, T., Shaik, S.M., Baufeld, C., Leibowitz, J., Zhang, X., Gottel, N., Zhang, X., Butovsky, O., Gilbert, J.A., et al. (2019). Sex-specific effects of microbiome perturbations on cerebral Aβ amyloidosis and microglia phenotypes. J. Exp. Med. *216*, 1542–1560.
67. Douglas, A.E. (2018). Which experimental systems should we use for human microbiome science? PLoS Biol. *16*, e2005245.
68. Douglas, A.E. (2019). Simple animal models for microbiome research. Nature Reviews Microbiology *17*, 764–775.
69. Erdman, S.E. (2021). Oxytocin and the microbiome. Current Opinion in Endocrine and Metabolic Research.
70. Erny, D., Hrabě de Angelis, A.L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., Keren-Shaul, H., Mahlakoiv, T., Jakobshagen, K., Buch, T., et al. (2015). Host microbiota constantly control maturation and function of microglia in the CNS. Nat. Neurosci. *18*, 965–977.
71. Fan, Y., and Pedersen, O. (2020). Gut microbiota in human metabolic health and disease. Nat. Rev. Microbiol.
72. Fang, P., Kazmi, S.A., Jameson, K.G., and Hsiao, E.Y. (2020). The Microbiome as a Modifier of Neurodegenerative Disease Risk. Cell Host Microbe *28*, 201–222.
73. Farzi, A., Fröhlich, E.E., and Holzer, P. (2018). Gut Microbiota and the Neuroendocrine System. Neurotherapeutics *15*, 5–22.
74. Figueroa-Romero, C., Guo, K., Murdock, B.J., Paez-Colasante, X., Bassis, C.M., Mikhail, K.A., Raue, K.D., Evans, M.C., Taubman, G.F., McDermott, A.J., et al. (2019). Temporal evolution of the microbiome, immune system and epigenome with disease progression in ALS mice. Dis. Model. Mech. *13*.
75. Fischer, C.N., Trautman, E.P., Crawford, J.M., Stabb, E.V., Handelsman, J., and Broderick, N.A. (2017). Metabolite exchange between microbiome members produces compounds that influence Drosophila behavior. Elife *6*.
76. Fontana, B.D., Müller, T.E., Cleal, M., de Abreu, M.S., Norton, W.H.J., Demin, K.A., Amstislavskaya, T.G., Petersen, E.V., Kalueff, A.V., Parker, M.O., et al. (2021). Using zebrafish (Danio rerio) models to understand the critical role of social interactions in mental health and wellbeing. Prog. Neurobiol. 101993. https://doi.org/10.1016/j.pneurobio.2021.101993.
77. Foster, J.A., Rinaman, L., and Cryan, J.F. (2017). Stress & the gut-brain axis: Regulation by the microbiome. Neurobiology of Stress *7*, 124–136.
78. Fülling, C., Dinan, T.G., and Cryan, J.F. (2019). Gut Microbe to Brain Signaling: What Happens in Vagus…. Neuron *101*, 998–1002.
79. Fung, T.C. (2020). The microbiota-immune axis as a central mediator of gut-brain communication. Neurobiol. Dis. *136*, 104714.
80. Fung, T.C., Olson, C.A., and Hsiao, E.Y. (2017). Interactions between the microbiota, immune and nervous systems in health and disease. Nat. Neurosci. *20*, 145–155.
81. Gaulke, C.A., Beaver, L.M., Armour, C.R., Humphreys, I.R., Barton, C.L., Tanguay, R.L., Ho, E., and Sharpton, T.J. (2020). An integrated gene catalog of the zebrafish gut microbiome reveals significant homology with mammalian microbiomes. bioRxiv. https://doi.org/10.1101/2020.06.15.153924.
82. Gehrig, J.L., Venkatesh, S., Chang, H.-W., Hibberd, M.C., Kung, V.L., Cheng, J., Chen, R.Y., Subramanian, S., Cowardin, C.A., Meier, M.F., et al. (2019). Effects of microbiota-directed foods in gnotobiotic animals and undernourished children. Science *365*.
83. Geng, Y., and Peterson, R.T. (2019). The zebrafish subcortical social brain as a model for studying social behavior disorders. Dis. Model. Mech. *12*.
84. Gheorghe, C.E., Ritz, N., Martin, J.A., Wardill, H.R., Cryan, J.F., and Clarke, G. (2021). Investigating causality with faecal microbiota transplantation in rodents: applications, recommendations and pitfalls. Gut Microbes.
85. Gill, S.R., Pop, M., DeBoy, R.T., Eckburg, P.B., Turnbaugh, P.J., Samuel, B.S., Gordon, J.I., Relman, D.A., Fraser-Liggett, C.M., and Nelson, K.E. (2006). Metagenomic Analysis of the Human Distal Gut Microbiome. Science *312*, 1355–1359.
86. Golubeva, A.V., Crampton, S., Desbonnet, L., Edge, D., O'Sullivan, O., Lomasney, K.W., Zhdanov, A.V., Crispie, F., Moloney, R.D., Borre, Y.E., et al. (2015). Prenatal stress-induced alterations in major physiological systems correlate with gut microbiota composition in adulthood. Psychoneuroendocrinology *60*, 58–74.
87. Golubeva, A.V., Joyce, S.A., Moloney, G., Burokas, A., Sherwin, E., Arboleya, S., Flynn, I., Khochanskiy, D., Moya-Pérez, A., Peterson, V., et al. (2017). Microbiota-related changes in bile acid & tryptophan metabolism are associated with gastrointestinal dysfunction in a mouse model of autism. EBioMedicine *24*, 166–178.
88. Gordon, J.I., and Klaenhammer, T.R. (2011). A rendezvous with our microbes. Proc. Natl. Acad. Sci. U. S. A. *108*,

4513–4515.
89. Govindan, J.A., Jayamani, E., Zhang, X., Mylonakis, E., and Ruvkun, G. (2015). Dialogue between E. coli free radical pathways and the mitochondria of C. elegans. Proc. Natl. Acad. Sci. U. S. A. *112*, 12456–12461.
90. Goya, M.E., Xue, F., Sampedro-Torres-Quevedo, C., Arnaouteli, S., Riquelme-Dominguez, L., Romanowski, A., Brydon, J., Ball, K.L., Stanley-Wall, N.R., and Doitsidou, M. (2020). Probiotic Bacillus subtilis Protects against α-Synuclein Aggregation in C. elegans. Cell Rep. *30*, 367-380.e7.
91. Gray, J.M., Karow, D.S., Lu, H., Chang, A.J., Chang, J.S., Ellis, R.E., Marletta, M.A., and Bargmann, C.I. (2004). Oxygen sensation and social feeding mediated by a C. elegans guanylate cyclase homologue. Nature *430*, 317–322.
92. Grenier, T., and Leulier, F. (2020). How commensal microbes shape the physiology of Drosophila melanogaster. Curr Opin Insect Sci *41*, 92–99.
93. Grinberg, M., Neuman, H., Ziv, O., Turjeman, S., Nosenko, R., and Koren, O. (2020). Antibiotics increase aggression behavior and aggression-related pheromones and receptors in Drosophila melanogaster.Biorxiv https://doi.org/10.1101/2020.09.22.307777.
94. Grinevich, V., and Neumann, I.D. (2020). Brain oxytocin: how puzzle stones from animal studies translate into psychiatry. Mol. Psychiatry.
95. Guida, F., Turco, F., Iannotta, M., De Gregorio, D., Palumbo, I., Sarnelli, G., Furiano, A., Napolitano, F., Boccella, S., Luongo, L., et al. (2018). Antibiotic-induced microbiota perturbation causes gut endocannabinoidome changes, hippocampal neuroglial reorganization and depression in mice. Brain Behav. Immun. *67*, 230–245.
96. Gur, T.L., Shay, L., Palkar, A.V., Fisher, S., Varaljay, V.A., Dowd, S., and Bailey, M.T. (2017). Prenatal stress affects placental cytokines and neurotrophins, commensal microbes, and anxiety-like behavior in adult female offspring. Brain Behav. Immun. *64*, 50–58.
97. Guthrie, L., Wolfson, S., and Kelly, L. (2019). The human gut chemical landscape predicts microbe-mediated biotransformation of foods and drugs. Elife *8*.
98. Hales, K.G., Korey, C.A., Larracuente, A.M., and Roberts, D.M. (2015). Genetics on the Fly: A Primer on the Drosophila Model System. Genetics *201*, 815–842.
99. Harach, T., Marungruang, N., Duthilleul, N., Cheatham, V., Mc Coy, K.D., Frisoni, G., Neher, J.J., Fåk, F., Jucker, M., Lasser, T., et al. (2017). Reduction of Abeta amyloid pathology in APPPS1 transgenic mice in the absence of gut microbiota. Sci. Rep. *7*.
100. Henriques, S.F., Dhakan, D.B., Serra, L., Francisco, A.P., Carvalho-Santos, Z., Baltazar, C., Elias, A.P., Anjos, M., Zhang, T., Maddocks, O.D.K., et al. (2020). Metabolic cross-feeding in imbalanced diets allows gut microbes to improve reproduction and alter host behaviour. Nat. Commun. *11*, 4236.
101. Hostinar, C.E., Sullivan, R.M., and Gunnar, M.R. (2014). Psychobiological mechanisms underlying the social buffering of the hypothalamic-pituitary-adrenocortical axis: a review of animal models and human studies across development. Psychol. Bull. *140*, 256–282.
102. 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., et al. (2013). Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell *155*, 1451–1463.
103. Hugenholtz, F., and de Vos, W.M. (2018). Mouse models for human intestinal microbiota research: a critical evaluation. Cell. Mol. Life Sci. *75*, 149–160.
104. Huo, R., Zeng, B., Zeng, L., Cheng, K., Li, B., Luo, Y., Wang, H., Zhou, C., Fang, L., Li, W., et al. (2017). Microbiota Modulate Anxiety-Like Behavior and Endocrine Abnormalities in Hypothalamic-Pituitary-Adrenal Axis. Front. Cell. Infect. Microbiol. *7*, 489.
105. Itskov, P.M., and Ribeiro, C. (2013). The dilemmas of the gourmet fly: the molecular and neuronal mechanisms of feeding and nutrient decision making in Drosophila. Front. Neurosci. *7*, 12.
106. Jaggar, M., Rea, K., Spichak, S., Dinan, T.G., and Cryan, J.F. (2020). You've got male: Sex and the microbiota-gut-brain axis across the lifespan. Front. Neuroendocrinol. *56*, 100815.
107. Jašarević, E., Howard, C.D., Morrison, K., Misic, A., Weinkopff, T., Scott, P., Hunter, C., Beiting, D., and Bale, T.L. (2018). The maternal vaginal microbiome partially mediates the effects of prenatal stress on offspring gut and hypothalamus. Nat. Neurosci. *21*, 1061–1071.
108. Jékely, G., Melzer, S., Beets, I., Kadow, I.C.G., Koene, J., Haddad, S., and Holden-Dye, L. (2018). The long and the short of it – a perspective on peptidergic regulation of circuits and behaviour. J. Exp. Biol. *221*, jeb166710.
109. Jia, Y., Jin, S., Hu, K., Geng, L., Han, C., Kang, R., Pang, Y., Ling, E., Tan, E.K., Pan, Y., et al. (2021). Gut microbiome modulates Drosophila aggression through octopamine signaling. Nat. Commun. *12*, 2698.
110. Joseph, R.M., and Carlson, J.R. (2015). Drosophila Chemoreceptors: A Molecular Interface Between the Chemical

World and the Brain. Trends Genet. *31*, 683–695.
111. Juster, R.-P., McEwen, B.S., and Lupien, S.J. (2010). Allostatic load biomarkers of chronic stress and impact on health and cognition. Neurosci. Biobehav. Rev. *35*, 2–16.
112. Keebaugh, E.S., Yamada, R., Obadia, B., Ludington, W.B., and Ja, W.W. (2018). Microbial Quantity Impacts Drosophila Nutrition, Development, and Lifespan. IScience *4*, 247–259.
113. Kelly, J.R., Borre, Y., O' Brien, C., Patterson, E., El Aidy, S., Deane, J., Kennedy, P.J., Beers, S., Scott, K., Moloney, G., et al. (2016). Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. J. Psychiatr. Res. *82*, 109–118.
114. Kim, B., Kanai, M.I., Oh, Y., Kyung, M., Kim, E.-K., Jang, I.-H., Lee, J.-H., Kim, S.-G., Suh, G.S.B., and Lee, W.-J. (2021). Response of the microbiome–gut–brain axis in Drosophila to amino acid deficit. Nature 1–5.
115. Kim, M.-S., Kim, Y., Choi, H., Kim, W., Park, S., Lee, D., Kim, D.K., Kim, H.J., Choi, H., Hyun, D.-W., et al. (2020). Transfer of a healthy microbiota reduces amyloid and tau pathology in an Alzheimer's disease animal model. Gut *69*, 283–294.
116. Kim, S., Kim, H., Yim, Y.S., Ha, S., Atarashi, K., Tan, T.G., Longman, R.S., Honda, K., Littman, D.R., Choi, G.B., et al. (2017). Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. Nature *549*, 528–532.
117. Kong, Y., Jiang, B., and Luo, X. (2018). Gut microbiota influences Alzheimer's disease pathogenesis by regulating acetate in Drosophila model. Future Microbiol. *13*, 1117–1128.
118. Koutzoumis, D.N., Vergara, M., Pino, J., Buddendorff, J., Khoshbouei, H., Mandel, R.J., and Torres, G.E. (2020). Alterations of the gut microbiota with antibiotics protects dopamine neuron loss and improve motor deficits in a pharmacological rodent model of Parkinson's disease. Exp. Neurol. *325*, 113159.
119. Lavasani, S., Dzhambazov, B., Nouri, M., Fåk, F., Buske, S., Molin, G., Thorlacius, H., Alenfall, J., Jeppsson, B., and Weström, B. (2010). A novel probiotic mixture exerts a therapeutic effect on experimental autoimmune encephalomyelitis mediated by IL-10 producing regulatory T cells. PLoS One *5*, e9009.
120. Leclercq, S., Mian, F.M., Stanisz, A.M., Bindels, L.B., Cambier, E., Ben-Amram, H., Koren, O., Forsythe, P., and Bienenstock, J. (2017). Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior. Nat. Commun. *8*, 15062.
121. Leclercq, S., Le Roy, T., Furgiuele, S., Coste, V., Bindels, L.B., Leyrolle, Q., Neyrinck, A.M., Quoilin, C., Amadieu, C., Petit, G., et al. (2020). Gut Microbiota-Induced Changes in β-Hydroxybutyrate Metabolism Are Linked to Altered Sociability and Depression in Alcohol Use Disorder. Cell Rep. *33*, 108238.
122. Lee, H.-J., Lee, K.-E., Kim, J.-K., and Kim, D.-H. (2019). Suppression of gut dysbiosis by Bifidobacterium longum alleviates cognitive decline in 5XFAD transgenic and aged mice. Sci. Rep. *9*, 11814.
123. Leftwich, P.T., Clarke, N.V.E., Hutchings, M.I., and Chapman, T. (2017). Gut microbiomes and reproductive isolation in Drosophila. Proc. Natl. Acad. Sci. U. S. A. *114*, 12767–12772.
124. Leitão-Gonçalves, R., Carvalho-Santos, Z., Francisco, A.P., Fioreze, G.T., Anjos, M., Baltazar, C., Elias, A.P., Itskov, P.M., Piper, M.D.W., and Ribeiro, C. (2017). Commensal bacteria and essential amino acids control food choice behavior and reproduction. PLoS Biol. *15*, e2000862.
125. Li, M., Zhao, L., Page-McCaw, P.S., and Chen, W. (2016). Zebrafish Genome Engineering Using the CRISPR–Cas9 System. Trends Genet. *32*, 815–827.
126. Liberti, J., and Engel, P. (2020). The gut microbiota — brain axis of insects. Current Opinion in Insect Science *39*, 6–13.
127. Liu, Y., and Forsythe, P. (2021). Vagotomy and insights into the microbiota-gut-brain axis. Neurosci. Res.
128. Liu, X., Li, X., Xia, B., Jin, X., Zou, Q., Zeng, Z., Zhao, W., Yan, S., Li, L., Yuan, S., et al. (2021). High-fiber diet mitigates maternal obesity-induced cognitive and social dysfunction in the offspring via gut-brain axis. Cell Metab.
129. Liu, Z., Kariya, M.J., Chute, C.D., Pribadi, A.K., Leinwand, S.G., Tong, A., Curran, K.P., Bose, N., Schroeder, F.C., Srinivasan, J., et al. (2018). Predator-secreted sulfolipids induce defensive responses in C. elegans. Nat. Commun. *9*, 1128.
130. Long-Smith, C., O'Riordan, K.J., Clarke, G., Stanton, C., Dinan, T.G., and Cryan, J.F. (2020). Microbiota-gut-brain axis: New therapeutic opportunities. Annu. Rev. Pharmacol. Toxicol. *60*, 477–502.
131. Luczynski, P., McVey Neufeld, K.-A., Oriach, C.S., Clarke, G., Dinan, T.G., and Cryan, J.F. (2016). Growing up in a Bubble: Using Germ-Free Animals to Assess the Influence of the Gut Microbiota on Brain and Behavior. Int. J. Neuropsychopharmacol. *19*.
132. Lyons, D.G., and Rihel, J. (2020). Sleep circuits and physiology in non-mammalian systems. Current Opinion in Physiology *15*, 245–255.
133. Macosko, E.Z., Pokala, N., Feinberg, E.H., Chalasani, S.H., Butcher, R.A., Clardy, J., and Bargmann, C.I. (2009). A

hub-and-spoke circuit drives pheromone attraction and social behaviour in C. elegans. Nature *458*, 1171–1175.
134. Maini Rekdal, V., Bess, E.N., Bisanz, J.E., Turnbaugh, P.J., and Balskus, E.P. (2019). Discovery and inhibition of an interspecies gut bacterial pathway for Levodopa metabolism. Science *364*.
135. Mangalam, A., Shahi, S.K., Luckey, D., Karau, M., Marietta, E., Luo, N., Choung, R.S., Ju, J., Sompallae, R., Gibson-Corley, K., et al. (2017). Human Gut-Derived Commensal Bacteria Suppress CNS Inflammatory and Demyelinating Disease. Cell Rep. *20*, 1269–1277.
136. Masuzzo, A., Montanari, M., Kurz, L., and Royet, J. (2020). How Bacteria Impact Host Nervous System and Behaviors: Lessons from Flies and Worms. Trends Neurosci.
137. Matty, M.A., and Chalasani, S.H. (2020). Microbial Mind Control. Cell Host Microbe *28*, 147–149.
138. Mayer, E.A., Knight, R., Mazmanian, S.K., Cryan, J.F., and Tillisch, K. (2014). Gut microbes and the brain: paradigm shift in neuroscience. J. Neurosci. *34*, 15490–15496.
139. McCarthy, M.M., Nugent, B.M., and Lenz, K.M. (2017). Neuroimmunology and neuroepigenetics in the establishment of sex differences in the brain. Nat. Rev. Neurosci. *18*, 471–484.
140. Mcfall-Ngai, M., Hadfield, M.G., Bosch, T.C.G., Carey, H.V., Domazet-Lo, T., Douglas, A.E., Dubilier, N., Eberl, G., Fukami, T., Gilbert, S.F., et al. (2013). Animals in a bacterial world, a new imperative for the life sciences. Proc. Natl. Acad. Sci. U. S. A. *110*, 3229–3236.
141. Medel-Matus, J.-S., Shin, D., Dorfman, E., Sankar, R., and Mazarati, A. (2018). Facilitation of kindling epileptogenesis by chronic stress may be mediated by intestinal microbiome. Epilepsia Open *3*, 290–294.
142. Medina-Rodriguez, E.M., Madorma, D., O'Connor, G., Mason, B.L., Han, D., Deo, S.K., Oppenheimer, M., Nemeroff, C.B., Trivedi, M.H., Daunert, S., et al. (2020). Identification of a Signaling Mechanism by Which the Microbiome Regulates Th17 Cell-Mediated Depressive-Like Behaviors in Mice. Am. J. Psychiatry *177*, 974–990.
143. Meisel, J.D., and Kim, D.H. (2014). Behavioral avoidance of pathogenic bacteria by Caenorhabditis elegans. Trends Immunol. *35*, 465–470.
144. Melancon, E., Gomez De La Torre Canny, S., Sichel, S., Kelly, M., Wiles, T.J., Rawls, J.F., Eisen, J.S., and Guillemin, K. (2017). Best practices for germ-free derivation and gnotobiotic zebrafish husbandry. Methods Cell Biol. *138*, 61–100.
145. Minter, M.R., Zhang, C., Leone, V., Ringus, D.L., Zhang, X., Oyler-Castrillo, P., Musch, M.W., Liao, F., Ward, J.F., Holtzman, D.M., et al. (2016). Antibiotic-induced perturbations in gut microbial diversity influences neuro-inflammation and amyloidosis in a murine model of Alzheimer's disease. Sci. Rep. *6*, 30028.
146. Morais, L.H., Schreiber, H.L., 4th, and Mazmanian, S.K. (2020a). The gut microbiota-brain axis in behaviour and brain disorders. Nat. Rev. Microbiol.
147. Morais, L.H., Golubeva, A.V., Moloney, G.M., Moya-Pérez, A., Ventura-Silva, A.P., Arboleya, S., Bastiaanssen, T.F.S., O'Sullivan, O., Rea, K., Borre, Y., et al. (2020b). Enduring Behavioral Effects Induced by Birth by Caesarean Section in the Mouse. Curr. Biol. *30*, 3761-3774.e6.
148. Nagpal, J., and Cryan, J.F. (2021). Host genetics, the microbiome & behaviour-a "Holobiont" perspective. Cell Res.
149. Nagpal, J., Herget, U., Choi, M.K., and Ryu, S. (2019). Anatomy, development, and plasticity of the neurosecretory hypothalamus in zebrafish. Cell Tissue Res. *375*, 5–22.
150. Needham, B.D., Kaddurah-Daouk, R., and Mazmanian, S.K. (2020). Gut microbial molecules in behavioural and neurodegenerative conditions. Nat. Rev. Neurosci.
151. Nestler, E.J., and Hyman, S.E. (2010). Animal models of neuropsychiatric disorders. Nat. Neurosci. *13*, 1161–1169.
152. Neufeld, K.M., Kang, N., Bienenstock, J., and Foster, J.A. (2011). Reduced anxiety-like behavior and central neurochemical change in germ-free mice: Behavior in germ-free mice. Neurogastroenterology & Motility *23*, 255-e119.
153. Newell, C., Bomhof, M.R., Reimer, R.A., Hittel, D.S., Rho, J.M., and Shearer, J. (2016). Ketogenic diet modifies the gut microbiota in a murine model of autism spectrum disorder. Molecular Autism *7*.
154. Nyholm, L., Koziol, A., Marcos, S., Botnen, A.B., Aizpurua, O., Gopalakrishnan, S., Limborg, M.T., Gilbert, M.T.P., and Alberdi, A. (2020). Holo-Omics: Integrated Host-Microbiota Multi-omics for Basic and Applied Biological Research. IScience *23*, 101414.
155. O'Connor, R., Moloney, G.M., Fulling, C., O'Riordan, K., Fitzgerald, P., Thomaz, B., Schellekens, H., Dinan, T.G., and Cryan, J.F. (2021). Maternal Antibiotic Administration During a Critical Developmental Window Has Enduring Neurobehavioural Effects in Offspring Mice. Behav. Brain Res. 113156.
156. Odekunle, E.A., and Elphick, M.R. (2020). Comparative and Evolutionary Physiology of Vasopressin/ Oxytocin-Type Neuropeptide Signaling in Invertebrates. Front. Endocrinol. *11*, 225.
157. O'Donnell, M.P., Fox, B.W., Chao, P.-H., Schroeder, F.C., and Sengupta, P. (2020). A neurotransmitter produced by gut bacteria modulates host sensory behaviour. Nature *583*, 415–420.

158. O'Leary, O.F., Ogbonnaya, E.S., Felice, D., Levone, B.R., C Conroy, L., Fitzgerald, P., Bravo, J.A., Forsythe, P., Bienenstock, J., Dinan, T.G., et al. (2018). The vagus nerve modulates BDNF expression and neurogenesis in the hippocampus. Eur. Neuropsychopharmacol. *28*, 307–316.
159. Olson, C.A., Vuong, H.E., Yano, J.M., Liang, Q.Y., Nusbaum, D.J., and Hsiao, E.Y. (2018). The Gut Microbiota Mediates the Anti-Seizure Effects of the Ketogenic Diet. Cell *173*, 1728-1741.e13.
160. O'Mahony, S.M., Marchesi, J.R., Scully, P., Codling, C., Ceolho, A.-M., Quigley, E.M.M., Cryan, J.F., and Dinan, T.G. (2009). Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. Biol. Psychiatry *65*, 263–267.
161. Pearson-Leary, J., Zhao, C., Bittinger, K., Eacret, D., Luz, S., Vigderman, A.S., Dayanim, G., and Bhatnagar, S. (2020). The gut microbiome regulates the increases in depressive-type behaviors and in inflammatory processes in the ventral hippocampus of stress vulnerable rats. Mol. Psychiatry *25*, 1068–1079.
162. Pham, L.N., Kanther, M., Semova, I., and Rawls, J.F. (2008). Methods for generating and colonizing gnotobiotic zebrafish. Nat. Protoc. *3*, 1862–1875.
163. Phelps, D., Brinkman, N.E., Keely, S.P., Anneken, E.M., Catron, T.R., Betancourt, D., Wood, C.E., Espenschied, S.T., Rawls, J.F., and Tal, T. (2017). Microbial colonization is required for normal neurobehavioral development in zebrafish. Sci. Rep. *7*, 11244.
164. Pryor, R., Norvaisas, P., Marinos, G., Best, L., Thingholm, L.B., Quintaneiro, L.M., De Haes, W., Esser, D., Waschina, S., Lujan, C., et al. (2019). Host-Microbe-Drug-Nutrient Screen Identifies Bacterial Effectors of Metformin Therapy. Cell *178*, 1299-1312.e29.
165. Pugin, B., Barcik, W., Westermann, P., Heider, A., Wawrzyniak, M., Hellings, P., Akdis, C.A., and O'Mahony, L. (2017). A wide diversity of bacteria from the human gut produces and degrades biogenic amines. Microb. Ecol. Health Dis. *28*, 1353881.
166. Pyndt Jørgensen, B., Krych, L., Pedersen, T.B., Plath, N., Redrobe, J.P., Hansen, A.K., Nielsen, D.S., Pedersen, C.S., Larsen, C., and Sørensen, D.B. (2015). Investigating the long-term effect of subchronic phencyclidine-treatment on novel object recognition and the association between the gut microbiota and behavior in the animal model of schizophrenia. Physiol. Behav. *141*, 32–39.
167. Qiao, H., Keesey, I.W., Hansson, B.S., and Knaden, M. (2019). Gut microbiota affects development and olfactory behavior in Drosophila melanogaster. The Journal of Experimental Biology *222*, jeb192500.
168. Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. Nature *464*, 59–65.
169. Ratsika, A., Codagnone, M.C., O'Mahony, S., Stanton, C., and Cryan, J.F. (2021). Priming for Life: Early Life Nutrition and the Microbiota-Gut-Brain Axis. Nutrients *13*, 423.
170. Rawls, J.F., Mahowald, M.A., Ley, R.E., and Gordon, J.I. (2006). Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. Cell *127*, 423–433.
171. Reddy, K.C., Hunter, R.C., Bhatla, N., Newman, D.K., and Kim, D.H. (2011). Caenorhabditis elegans NPR-1–mediated behaviors are suppressed in the presence of mucoid bacteria. Proc. Natl. Acad. Sci. U. S. A. *108*, 12887–12892.
172. Rhoades, J.L., Nelson, J.C., Nwabudike, I., Yu, S.K., McLachlan, I.G., Madan, G.K., Abebe, E., Powers, J.R., Colón-Ramos, D.A., and Flavell, S.W. (2019). ASICs Mediate Food Responses in an Enteric Serotonergic Neuron that Controls Foraging Behaviors. Cell *176*, 85-97.e14.
173. Robinson, K.J., Bosch, O.J., Levkowitz, G., Busch, K.E., Jarman, A.P., and Ludwig, M. (2019). Social creatures: Model animal systems for studying the neuroendocrine mechanisms of social behaviour. Journal of Neuroendocrinology *31*.
174. Roeselers, G., Mittge, E.K., Stephens, W.Z., Parichy, D.M., Cavanaugh, C.M., Guillemin, K., and Rawls, J.F. (2011). Evidence for a core gut microbiota in the zebrafish. ISME J. *5*, 1595–1608.
175. Sampson, T.R., and Mazmanian, S.K. (2015). Control of brain development, function, and behavior by the microbiome. Cell Host Microbe *17*, 565–576.
176. 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., et al. (2016). Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. Cell *167*, 1469-1480.e12.
177. Sampson, T.R., Challis, C., Jain, N., Moiseyenko, A., Ladinsky, M.S., Shastri, G.G., Thron, T., Needham, B.D., Horvath, I., Debelius, J.W., et al. (2020). A gut bacterial amyloid promotes a-synuclein aggregation and motor impairment in mice. Elife *9*.
178. Samuel, B.S., Rowedder, H., Braendle, C., Félix, M.-A., and Ruvkun, G. (2016). Caenorhabditis elegans responses

to bacteria from its natural habitats. Proc. Natl. Acad. Sci. U. S. A. *113*, E3941-9.
179. Sandi, C., and Haller, J. (2015). Stress and the social brain: behavioural effects and neurobiological mechanisms. Nat. Rev. Neurosci. *16*, 290–304.
180. Schneider, A.C.R., Rico, E.P., de Oliveira, D.L., Rosemberg, D.B., Guizzo, R., Meurer, F., and da Silveira, T.R. (2016). Lactobacillus rhamnosus GG Effect on Behavior of Zebrafish During Chronic Ethanol Exposure. Biores. Open Access *5*, 1–5.
181. Schnörr, S.J., Steenbergen, P.J., Richardson, M.K., and Champagne, D.L. (2012). Measuring thigmotaxis in larval zebrafish. Behav. Brain Res. *228*, 367–374.
182. Schretter, C.E., Vielmetter, J., Bartos, I., Marka, Z., Marka, S., Argade, S., and Mazmanian, S.K. (2018). A gut microbial factor modulates locomotor behaviour in Drosophila. Nature *563*, 402–406.
183. Schulenburg, H., and Félix, M.A. (2017). The natural biotic environment of Caenorhabditis elegans. Genetics *206*, 55–86.
184. Scott, T.A., Quintaneiro, L.M., Norvaisas, P., Lui, P.P., Wilson, M.P., Leung, K.Y., Herrera-Dominguez, L., Sudiwala, S., Pessia, A., Clayton, P.T., et al. (2017). Host-Microbe Co-metabolism Dictates Cancer Drug Efficacy in C. elegans. Cell *169*, 442-456.e18.
185. Selkrig, J., Mohammad, F., Ng, S.H., Chua, J.Y., Tumkaya, T., Ho, J., Chiang, Y.N., Rieger, D., Pettersson, S., Helfrich-Förster, C., et al. (2018). The Drosophila microbiome has a limited influence on sleep, activity, and courtship behaviors. Sci. Rep. *8*, 10646.
186. Sender, R., Fuchs, S., and Milo, R. (2016). Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans. Cell *164*, 337–340.
187. Sgritta, M., Dooling, S.W., Buffington, S.A., Momin, E.N., Francis, M.B., Britton, R.A., and Costa-Mattioli, M. (2019). Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in Mouse Models of Autism Spectrum Disorder. Neuron *101*, 246-259.e6.
188. Shan Xu, C., Januszewski, M., Lu, Z., Takemura, S.-Y., Hayworth, K.J., Huang, G., Shinomiya, K., Maitin-Shepard, J., Ackerman, D., Berg, S., et al. (2020). A Connectome of the Adult Drosophila Central Brain. Biorxiv
189. Sharon, G., Segal, D., Ringo, J.M., Hefetz, A., Zilber-Rosenberg, I., and Rosenberg, E. (2010). Commensal bacteria play a role in mating preference of Drosophila melanogaster. Proc. Natl. Acad. Sci. U. S. A. *107*, 20051–20056.
190. Sharon, G., Sampson, T.R., Geschwind, D.H., and Mazmanian, S.K. (2016). The Central Nervous System and the Gut Microbiome. Cell *167*, 915–932.
191. Sharon, G., Cruz, N.J., Kang, D.-W., Gandal, M.J., Wang, B., Kim, Y.-M., Zink, E.M., Casey, C.P., Taylor, B.C., Lane, C.J., et al. (2019). Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice. Cell *177*, 1600-1618.e17.
192. Sherwin, E., Bordenstein, S.R., Quinn, J.L., Dinan, T.G., and Cryan, J.F. (2019). Microbiota and the social brain. Science *366*.
193. Shin, S.C., Kim, S.-H., You, H., Kim, B., Kim, A.C., Lee, K.-A., Yoon, J.-H., Ryu, J.-H., and Lee, W.-J. (2011). Drosophila microbiome modulates host developmental and metabolic homeostasis via insulin signaling. Science *334*, 670–674.
194. Silva, Y.P., Bernardi, A., and Frozza, R.L. (2020). The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. Front. Endocrinol. *11*, 25.
195. Singh, J., and Aballay, A. (2020). Neural control of behavioral and molecular defenses in C. elegans. Curr. Opin. Neurobiol. *62*, 34–40.
196. Sokolowski, M.B. (2001). Drosophila: genetics meets behaviour. Nat. Rev. Genet. *2*, 879–890.
197. Sokolowski, M.B. (2010). Social Interactions in “Simple” Model Systems. Neuron *65*, 780–794.
198. Sommer, F., and Bäckhed, F. (2013). The gut microbiota — masters of host development and physiology. Nat. Rev. Microbiol. *11*, 227–238.
199. Staubach, F., Baines, J.F., Künzel, S., Bik, E.M., and Petrov, D.A. (2013). Host species and environmental effects on bacterial communities associated with Drosophila in the laboratory and in the natural environment. PLoS One *8*, e70749.
200. Stewart, A.M., Ullmann, J.F.P., Norton, W.H.J., Parker, M.O., Brennan, C.H., Gerlai, R., and Kalueff, A.V. (2015). Molecular psychiatry of zebrafish. Mol. Psychiatry *20*, 2–17.
201. Strandwitz, P. (2018). Neurotransmitter modulation by the gut microbiota. Brain Res. *1693*, 128–133.
202. Strandwitz, P., Kim, K.H., Terekhova, D., Liu, J.K., Sharma, A., Levering, J., McDonald, D., Dietrich, D., Ramadhar, T.R., Lekbua, A., et al. (2019). GABA-modulating bacteria of the human gut microbiota. Nat Microbiol *4*, 396–403.
203. Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.N., Kubo, C., and Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J. Physiol. *558*,

263–275.
204. Sun, J., Xu, J., Ling, Y., Wang, F., Gong, T., Yang, C., Ye, S., Ye, K., Wei, D., Song, Z., et al. (2019). Fecal microbiota transplantation alleviated Alzheimer's disease-like pathogenesis in APP/PS1 transgenic mice. Transl. Psychiatry *9*, 189.
205. Sun, M.-F., Zhu, Y.-L., Zhou, Z.-L., Jia, X.-B., Xu, Y.-D., Yang, Q., Cui, C., and Shen, Y.-Q. (2018). Neuroprotective effects of fecal microbiota transplantation on MPTP-induced Parkinson's disease mice: Gut microbiota, glial reaction and TLR4/TNF-α signaling pathway. Brain Behav. Immun. *70*, 48–60.
206. Swann, J.R., Spitzer, S.O., and Diaz Heijtz, R. (2020). Developmental Signatures of Microbiota-Derived Metabolites in the Mouse Brain. Metabolites *10*.
207. Tabouy, L., Getselter, D., Ziv, O., Karpuj, M., Tabouy, T., Lukic, I., Maayouf, R., Werbner, N., Ben-Amram, H., Nuriel-Ohayon, M., et al. (2018). Dysbiosis of microbiome and probiotic treatment in a genetic model of autism spectrum disorders. Brain Behav. Immun. *73*, 310–319.
208. Taylor, V.H. (2019). The microbiome and mental health: Hope or hype? J. Psychiatry Neurosci. *44*, 219–222.
209. Tengeler, A.C., Dam, S.A., Wiesmann, M., Naaijen, J., van Bodegom, M., Belzer, C., Dederen, P.J., Verweij, V., Franke, B., Kozicz, T., et al. (2020). Gut microbiota from persons with attention-deficit/hyperactivity disorder affects the brain in mice. Microbiome *8*, 44.
210. de Theije, C.G.M., Wopereis, H., Ramadan, M., van Eijndthoven, T., Lambert, J., Knol, J., Garssen, J., Kraneveld, A.D., and Oozeer, R. (2014). Altered gut microbiota and activity in a murine model of autism spectrum disorders. Brain Behav. Immun. *37*, 197–206.
211. Theis, K.R., Dheilly, N.M., Klassen, J.L., Brucker, R.M., Baines, J.F., Bosch, T.C.G., Cryan, J.F., Gilbert, S.F., Goodnight, C.J., Lloyd, E.A., et al. (2016). Getting the Hologenome Concept Right: an Eco-Evolutionary Framework for Hosts and Their Microbiomes. MSystems *1*.
212. Tian, P., O'Riordan, K.J., Lee, Y.-K., Wang, G., Zhao, J., Zhang, H., Cryan, J.F., and Chen, W. (2020). Towards a psychobiotic therapy for depression: Bifidobacterium breve CCFM1025 reverses chronic stress-induced depressive symptoms and gut microbial abnormalities in mice. Neurobiol Stress *12*, 100216.
213. Urrutia, A., García-Angulo, V.A., Fuentes, A., Caneo, M., Legüe, M., Urquiza, S., Delgado, S.E., Ugalde, J., Burdisso, P., and Calixto, A. (2020). Bacterially produced metabolites protect C. elegans neurons from degeneration. PLoS Biol. *18*, e3000638.
214. Valcarce, D.G., Martínez-Vázquez, J.M., Riesco, M.F., and Robles, V. (2020). Probiotics reduce anxiety-related behavior in zebrafish. Heliyon *6*, e03973.
215. Valles-Colomer, M., Falony, G., Darzi, Y., Tigchelaar, E.F., Wang, J., Tito, R.Y., Schiweck, C., Kurilshikov, A., Joossens, M., Wijmenga, C., et al. (2019). The neuroactive potential of the human gut microbiota in quality of life and depression. Nature Microbiology *4*, 623–632.
216. Vanwalleghem, G.C., Ahrens, M.B., and Scott, E.K. (2018). Integrative whole-brain neuroscience in larval zebrafish. Curr. Opin. Neurobiol. *50*, 136–145.
217. Vazquez, E., Barranco, A., Ramirez, M., Gruart, A., Delgado-Garcia, J.M., Jimenez, M.L., Buck, R., and Rueda, R. (2016). Dietary 2'-Fucosyllactose Enhances Operant Conditioning and Long-Term Potentiation via Gut-Brain Communication through the Vagus Nerve in Rodents. PLoS One *11*, e0166070.
218. Vernier, C.L., Chin, I.M., Adu-Oppong, B., Krupp, J.J., Levine, J., Dantas, G., and Ben-Shahar, Y. (2020). The gut microbiome defines social group membership in honey bee colonies. Sci Adv *6*.
219. Vuong, H.E., Yano, J.M., Fung, T.C., and Hsiao, E.Y. (2017). The Microbiome and Host Behavior. Annu. Rev. Neurosci. *40*, annurev-neuro-072116-031347.
220. Vuong, H.E., Pronovost, G.N., Williams, D.W., Coley, E.J.L., Siegler, E.L., Qiu, A., Kazantsev, M., Wilson, C.J., Rendon, T., and Hsiao, E.Y. (2020). The maternal microbiome modulates fetal neurodevelopment in mice. Nature *586*, 281–286.
221. Wakade, C., Chong, R., Bradley, E., Thomas, B., and Morgan, J. (2014). Upregulation of GPR109A in Parkinson's disease. PLoS One *9*, e109818.
222. Walter, J., Armet, A.M., Finlay, B.B., and Shanahan, F. (2020). Establishing or Exaggerating Causality for the Gut Microbiome: Lessons from Human Microbiota-Associated Rodents. Cell *180*, 221–232.
223. Wang, G., Rajpurohit, S.K., Delaspre, F., Walker, S.L., White, D.T., Ceasrine, A., Kuruvilla, R., Li, R.-J., Shim, J.S., Liu, J.O., et al. (2015). First quantitative high-throughput screen in zebrafish identifies novel pathways for increasing pancreatic β-cell mass. Elife *4*.
224. Wang, X., Zheng, Y., Zhang, Y., Li, J., Zhang, H., and Wang, H. (2016). Effects of β-diketone antibiotic mixtures on behavior of zebrafish (Danio rerio). Chemosphere *144*, 2195–2205.
225. Watson, E., Macneil, L.T., Ritter, A.D., Yilmaz, L.S., Rosebrock, A.P., Caudy, A.A., and Walhout, A.J.M. (2014).

Interspecies systems biology uncovers metabolites affecting C. elegans gene expression and life history traits. Cell *156*, 759–770.
226. de Weerth, C. (2017). Do bacteria shape our development? Crosstalk between intestinal microbiota and HPA axis. Neurosci. Biobehav. Rev. *83*, 458–471.
227. Wendelaar Bonga, S.E. (1997). The stress response in fish. Physiol. Rev. *77*, 591–625.
228. Westfall, S., Lomis, N., and Prakash, S. (2019). A novel synbiotic delays Alzheimer's disease onset via combinatorial gut-brain-axis signaling in Drosophila melanogaster. PLoS One *14*, e0214985.
229. Wong, A.C.-N., Chaston, J.M., and Douglas, A.E. (2013). The inconstant gut microbiota of Drosophila species revealed by 16S rRNA gene analysis. ISME J. *7*, 1922–1932.
230. Wong, A.C.-N., Dobson, A.J., and Douglas, A.E. (2014). Gut microbiota dictates the metabolic response of Drosophila to diet. J. Exp. Biol. *217*, 1894–1901.
231. Wong, A.C.-N., Luo, Y., Jing, X., Franzenburg, S., Bost, A., and Douglas, A.E. (2015). The Host as the Driver of the Microbiota in the Gut and External Environment of Drosophila melanogaster. Appl. Environ. Microbiol. *81*, 6232–6240.
232. Wong, A.C.-N., Wang, Q.-P., Morimoto, J., Senior, A.M., Lihoreau, M., Gregory Neely, G., Simpson, S.J., and Ponton, F. (2017). Gut Microbiota Modifies Olfactory-Guided Microbial Preferences and Foraging Decisions in Drosophila. Current Biology *27*, 2397-2404.e4.
233. Worthy, S.E., Haynes, L., Chambers, M., Bethune, D., Kan, E., Chung, K., Ota, R., Taylor, C.J., and Glater, E.E. (2018). Identification of attractive odorants released by preferred bacterial food found in the natural habitats of C. elegans. PLOS ONE *13*, e0201158.
234. Wu, W.-L., Adame, M.D., Liou, C.-W., Barlow, J.T., Lai, T.-T., Sharon, G., Schretter, C.E., Needham, B.D., Wang, M.I., Tang, W., et al. (2021). Microbiota regulate social behaviour via stress response neurons in the brain. Nature 595, 409–414. https://doi.org/10.1038/s41586-021-03669-y.
235. Wu, S.-C., Cao, Z.-S., Chang, K.-M., and Juang, J.-L. (2017). Intestinal microbial dysbiosis aggravates the progression of Alzheimer's disease in Drosophila. Nat. Commun. *8*, 24.
236. Xiao, R., Chun, L., Ronan, E.A., Friedman, D.I., Liu, J., and Xu, X.Z.S. (2015). RNAi Interrogation of Dietary Modulation of Development, Metabolism, Behavior, and Aging in C. elegans. Cell Rep. *11*, 1123–1133.
237. Xu, S.C., Januszewski, M., Lu, Z., Takemura, S.-Y., Hayworth, K.J., Huang, G., Shinomiya, K., Maitin-Shepard, J., Ackerman, D., Berg, S., et al. (2020a). A Connectome of the Adult Drosophila Central Brain. bioRxiv. https://doi.org/ 10.1101/2020.01.21.911859.
238. Xu, Y., Xie, M., Xue, J., Xiang, L., Li, Y., Xiao, J., Xiao, G., and Wang, H.-L. (2020). EGCG ameliorates neuronal and behavioral defects by remodeling gut microbiota and TotM expression in Drosophila models of Parkinson's disease. FASEB J. *34*, 5931–5950.
239. Yang, Z., Li, J., Gui, X., Shi, X., Bao, Z., Han, H., and Li, M.D. (2020). Updated review of research on the gut microbiota and their relation to depression in animals and human beings. Mol. Psychiatry *25*, 2759–2772.
240. Ye, L., Bae, M., Cassilly, C.D., Jabba, S.V., Thorpe, D.W., Martin, A.M., Lu, H.-Y., Wang, J., Thompson, J.D., Lickwar, C.R., et al. (2020). Enteroendocrine cells sense bacterial tryptophan catabolites to activate enteric and vagal neuronal pathways. Cell Host Microbe.
241. Zhang, F., Berg, M., Dierking, K., Félix, M.A., Shapira, M., Samuel, B.S., and Schulenburg, H. (2017a). Caenorhabditis elegans as a model for microbiome research. Front. Microbiol. *8*.
242. Zhang, Y.-G., Wu, S., Yi, J., Xia, Y., Jin, D., Zhou, J., and Sun, J. (2017b). Target Intestinal Microbiota to Alleviate Disease Progression in Amyotrophic Lateral Sclerosis. Clin. Ther. *39*, 322–336.
243. Zheng, P., Zeng, B., Zhou, C., Liu, M., Fang, Z., Xu, X., Zeng, L., Chen, J., Fan, S., Du, X., et al. (2016). Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. Mol. Psychiatry *21*, 786–796.
244. Zheng, P., Zeng, B., Liu, M., Chen, J., Pan, J., Han, Y., Liu, Y., Cheng, K., Zhou, C., Wang, H., et al. (2019). The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice. Sci Adv *5*, eaau8317.

Table 1: Selected Examples of Microbiota-Gut-Brain axis interactions in Neurobehavioural Disorders in Preclinical Rodent Models.

Disorder	Model/Intervention	Microbiota-led effects on host brain and behaviour	References
Autism spectrum disorder (ASD)			
	Maternal immune activation (MIA) with poly(I:C)	*B. fragilis* reversed hyperactivity and anxiety-like behaviour, but not the social deficits. MIA-related abnormal behaviours were associated with the presence of segmented filamentous bacteria that induce intestinal TH17 cells.	(Hsiao et al., 2013; Kim et al., 2017)
	BTBR $T^{+}Itpr3^{tf}/J$	Administration of *L. reuteri* improved social deficits. Microbial metabolite - 5-aminovaleric acid (5AV) reduced neuronal excitability in the prefrontal cortex. Specific reduction in bile-metabolizing *Bifidobacterium* and *Blautia* species and *L. reuteri* levels	(Coretti et al., 2017; Golubeva et al., 2017; Newell et al., 2016; Sgritta et al., 2019; Sharon et al., 2019)
	In-utero valproic acid (VPA)	Reduced levels of serotonin in the prefrontal cortex and amygdala of VPA in utero-exposed males. *L. reuteri* ameliorated the social deficits in VPA mice.	(Sgritta et al., 2019; de Theije et al., 2014)
	Maternal high fat-diet (MHFD)	*L. reuteri* restored oxytocin levels, social reward mediated synaptic potentiation in ventral tegmental area (VTA), and social deficits in MHFD offspring.	(Buffington et al., 2016)
	shank3b$^{-/-}$	*L. reuteri* rescued social deficits and ventral tegmental area (VTA) synaptic plasticity, but not inmice lacking oxytocin receptor in dopaminergic neurons. The rescue of social deficits was dependent on the vagus nerve, specific to male mice while a decrease in repetitive behaviours was found in both male and female mice.	(Sgritta et al., 2019; Tabouy et al., 2018)
	cntnap2$^{-/-}$	*L. reuteri* and microbe-induced metabolite (BH4) rescue social deficits but not hyperactivity. Also improve oxytocin levels and VTA synaptic transmission.	(Buffington et al., 2021)
	FMT from ASD individuals into GF mice	Mice colonized with ASD microbiota showed repetitive behaviour and social deficits, and their brains showed alternative splicing of ASD-relevant genes. Microbial metabolites - 5AV and Taurine improved ASD deficits.	(Sharon et al., 2019)
Schizophrenia			
	Subchronic phencyclidine (subPCP) treatment	Impaired novel object recognition. Correlation between microbiome composition profiles and memory performance. Administration of ampicillin removed memory deficit induced by subPCP treatment.	(Pyndt Jørgensen et al., 2015)
	FMT from Schizophrenia patients in GF mice	Lower glutamate and increased glutamine and GABA in the hippocampus, and SCZ-relevant behaviours. Aerococcaceae and Rikenellaceae enabled complete distinction of SCZ microbiota recipient mice from controls.	(Zheng et al., 2019)
Attention deficit hyperactivity disorder			
	FMT from ADHD individuals into GF male mice	Recipient mice showed reduced structural integrity of both gray and white matter areas (i.e., hippocampus, internal capsule) and increased anxiety. White matter integrity was positively correlated withthe relative abundance of *Eubacterium and Holdemania*.	(Tengeler et al., 2020)
Epilepsy			
	6-Hz-induced seizure model of refractory epilepsy and Kcna1$^{-/-}$ model	Ketogenic diet, with notable increases in *Parabacteroides and Akkermansia,* provided protection from seizures. Changes in metabolome from hippocampus correlated with seizure protection. Decreased levels of systemic gamma-glutamylated amino acids and increased hippocampal GABA/glutamate observed.	(Olson et al., 2018)

	Chronic restraint stress model	Chronic stress and cecal microbiota transplanted from stressed to control rats hastened the progression and increased the duration of kindled seizures. Microbiota from control animals transplanted to chronically stressed rats, acted against proepileptic effects of restraint stress.	(Medel-Matus et al., 2018)
	WAG/Rij rat model	FMT, from both Wistar and ethosuximide-treated WAG/Rij donor rats to WAG/Rij rats, reduced the number and duration of seizures.	(Citraro et al., 2021)
Alzheimer's disease			
	Aβ precursor protein (APP), presenilin 1 ($PS1_{\Delta E9}$) APP/PS1 model	Antibiotic treatment resulted in decreased Aβ plaque deposition, elevated soluble Aβ, changes in the levels of circulating cytokines and microglial morphology, and attenuation of plaque-localised glial reactivity. Expansion of *Akkermansia* and *Lachnospiraceae* observed.	(Minter et al., 2016)
	Aβ precursor protein (APP), presenilin 1 ($PS1_{L166P}$) APP/PS1 model	Reduction of Aβ levels in brain and blood, as well as cortical neuroinflammation in GF-APP/PS1 mice as compared to conventionally raised ones. Colonization of GF APP/PS1 mice with microbiota from conventionally raised enhanced cerebral Aβ pathology.	(Harach et al., 2017)
	5xFAD model	*Bifidobacterium longum* (NK46) attenuated cognitive impairments, suppressed amyloid-β accumulation and NF-κB activation in the hippocampus, and suppressed the infiltration of activated microglia and apoptotic neurons into the hippocampus.	(Lee et al., 2019)
	FMT into $APP/PS1_{\Delta E9}$ model	FMT improved cognitive deficits, decreased deposition of amyloid-β in brain, reduced phosphorylation of tau protein, and increased postsynaptic density protein 95 (PSD-95) and synapsin I expression. FMT decreased the abundance of *Proteobacteria* and *Verrucomicrobia* and increased Bacteroidetes.	(Sun et al., 2019)
	$APP/PS1_{L166P}$ model	Antibiotic administration reduced amyloid-β pathology and alterated microglial morphology in male but not in female APP/PS mice. FMT from age-matched APP/PS male mice into antibiotic-treated male partially restored amyloid-β pathology and microglial morphology.	(Dodiya et al., 2019)
	$ADLP^{APT}$ transgenic mouse model of AD	FMT from WT mice alleviated Aβ deposition, memory deficits, tau pathology, and reactive gliosis in $ADLP^{APT}$ mice.	(Kim et al., 2020)
Parkinson's disease			
	FMT from PD patients to GF wildtype and alpha-synuclein (AS) mice.	Microbiota from PD patients enhanced motor deficits in AS animals in comparison to microbiota from healthy individuals. Increased abundance of *Proteus*, *Bilophila*, and *Roseburia* species in mice colonised with microbiota from PD donors	(Sampson et al., 2016)
	Thy-1-human AS mice	Reduced motor and gastrointestinal dysfunction, microglia activation and AS aggregation in GF AS mice. SCFA (acetate, propionate, butyrate) administration to GF AS mice promoted neuroinflammation and motor symptoms.	(Sampson et al., 2016)
	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model and FMT treatment	FMT from WT mice to MPTP mice alleviated motor impairment, increased striatal DA and 5-HT content, decreased the microglia and astrocyte activation in substantia nigra, decreased TLR4/TNF-alpha signaling in gut and brain, and reduced fecal SCFAs. FMT from MPTP to normal mice induced impaired motor function and striatal neurotransmission.	(Sun et al., 2018)
	Thy-1-human alpha-synuclein mice	Colonization with curli-producing *E. coli* exacerbated AS-induced deficits in behaviour, including motor and intestinal impairments. Further observations include increased microglial activation, elevated levels of proinflammatory cytokines interleukin 6 (IL-6) and tumor necrosis factor alpha (TNFa) in brain-derived CD11b+ cells, and rise in cytokine levels in striatum as well as midbrain.	(Sampson et al., 2020)

	Exposure to *E. coli* producing the extracellular bacterial amyloid protein curli in aged Fischer 344 Rats.	Increased neuronal alpha-synuclein (AS) deposition in brain and gut, increased brain expression of IL6, TLR2 and TNF, and elevated microgliosis and astrogliosis in comparison to rats administered vehicle or mutant bacteria unable to synthesize curli.	(Chen et al., 2016)
	6-hydroxydopamine (6-OHDA) rat model	Antibiotic administration attenuated motor dysfunction, TH-positive neuron loss, and expression of proinflammatory markers in striatum.	(Koutzoumis et al., 2020)
Multiple Sclerosis			
	Experimental autoimmune encephalomyelitis (EAE) model of MS.	Mixture of *L. paracasei* DSM13434, *L. plantarum* DSM15312 and DSM15313 inhibited the progression and improved the histological and clinical defcicits of EAE. The suppression correlated with reduction of pro-inflammatory Th1 and Th17 cytokines followed by IL-10 induction in mesenteric lymph nodes.	(Lavasani et al., 2010)
	Relapsing–remitting (RR) mouse model of spontaneously developing EAE	Using GF mice, microbiota was shown to be required for the development of spontaneous EAE in a process driven by myelin specific CD4+ T cells.	(Berer et al., 2011)
	FMT from MS patients to GF RR model of EAE	Increased incidence of spontaneous EAE and decreased IL-10 generation in RR Mice colonized with microbiota from MS-Affected Twins.	(Berer et al., 2017)
	FMT from MS patients to GF EAE model	More severe symptoms of EAE and decreased proportions of IL-10+ Tregs in mice receiving FMT from MS patients as compared to those from healthy controls.	(Cekanaviciute et al., 2017)
	EAE in HLA-DR3.DQ8 transgenic model	*Prevotella histicola* decreased CNS inflammation and demyelination through an increase in CD4+ FoxP3+ Tregs and a reduction in proinflammatory Th1 and Th17 cells. *P. histicola* exposure replenished the abundance of *Prevotella, Sutterella*, and *Lactobacillus*.	(Mangalam et al., 2017)
	Colonization of MS-associated *Akkermansia* strains to MOG/C57 model of EAE	A*kkermansia* isolated from MS patients improved EAE deficits, which was associated with a decrease in RORγt+ and IL-17–producing γδ T cells.	(Cox et al., 2021)
Amyotrophic lateral sclerosis (ALS)			
	$SOD1^{G93A}$	Administration of butyrate recovered gut integrity, gut microbiota diversity and prolonged life span in comparison to control mice.	(Zhang et al., 2017)
	$SOD1^{G93A}$	*Akkermansia muciniphila* colonization increased spinal cord cellular number, improved motor function and prolonged the lifespan. The associated metabolite Nicotinamide significantly improved motor performance.	(Blacher et al., 2019)
	$SOD1^{G93A}$	Microbiota alteration prior to the onset of motor dysfunction and muscle atrophy. Leukocyte infiltration into CNS tissue, elevated CNS myeloid activation and maturation accompanied by altered global brain 5hmC levels. Specific correlations observed among microbiome, epigenome and immune changes.	(Figueroa-Romero et al., 2019)
	C9orf72 loss-of-function mice	Antibiotic treatment and FMT in ALS mice modulated spinal cord neuroinflammation including microglial activation and neutrophil infiltration.	(Burberry et al., 2020)
Depression and anxiety disorders			
	Prenatal Stress and Maternal Separation	Prenatal stress led to alterations in microbiota community structure, with reduction in *Lactobacillus*. Associated with increased IL-1b *in utero*, exaggerated HPA axis response, reduction in brain-derived neurotrophic factor (BDNF), and long-term alterations in both behavior and microbiome in offspring. Partial mediation of prenatal stress-induced effects on hypothalamic gene expression in adult male mice by maternal vaginal microbiota transfer Plasma	(Golubeva et al., 2015; Gur et al., 2017; Jašarević et al., 2018; O'Mahony et al., 2009)

		corticosterone and visceral sensation were increased in the maternally separated rats showing altered microbiota.	
	FMT from patients suffering from depression / irritable bowel syndrome (IBS) and anxiety into microbiota-deficient rats and GF mice	Mice transplanted with microbiota from depressed and IBS/anxiety patients showed depression and anxiety-like behavior, respectively. Low-grade inflammation and immune activation in the gut also observed. FMT in rats promoted anhedonia and anxiety-like behaviours, and changes in tryptophan metabolism.	(De Palma et al., 2017; Kelly et al., 2016; Zheng et al., 2016)
	Microbiota disruption with antibiotic cocktail	Depressive-like behaviour along with changes in BDNF/TrkB signalling, TRPV1 phosphorylation, neuronal activity in the hippocampus, microglia activation states and endocannabinoid levels in the gut. Subsequent *Lactobacillus casei DG* administration reversed most of these gut inflammatory, behavioural, and neurochemical changes. *Lachnospiraceae* correlated with the behavioural alterations observed in antibiotic treated mice.	(Guida et al., 2018)
	Social defeat	Increased microglial density and IL-1β levels in the ventral hippocampus, and depressive-like behaviors in naive rats receiving FMT from vulnerable rats compared to FMT from resilient rats, non-stressed control rats, or vehicle-treated rats. Significant correlation between corticosterone levels, aspects of immune and behaviour response, and degree of microbiome compositional change over time.	(Bailey et al., 2011; Bastiaanssen et al., 2021; Pearson-Leary et al., 2020)
	Chronic unpredictable mild stress	*Bifidobacterium breve CCFM1025* administration decreased depression- and anxiety-like behaviors, elevated BDNF expression, attenuated hyperactive hypothalamic-pituitary-adrenal response and inflammation, down-regulation of pCREB-c-Fos pathway observed.	(Tian et al., 2020)

Figure 1

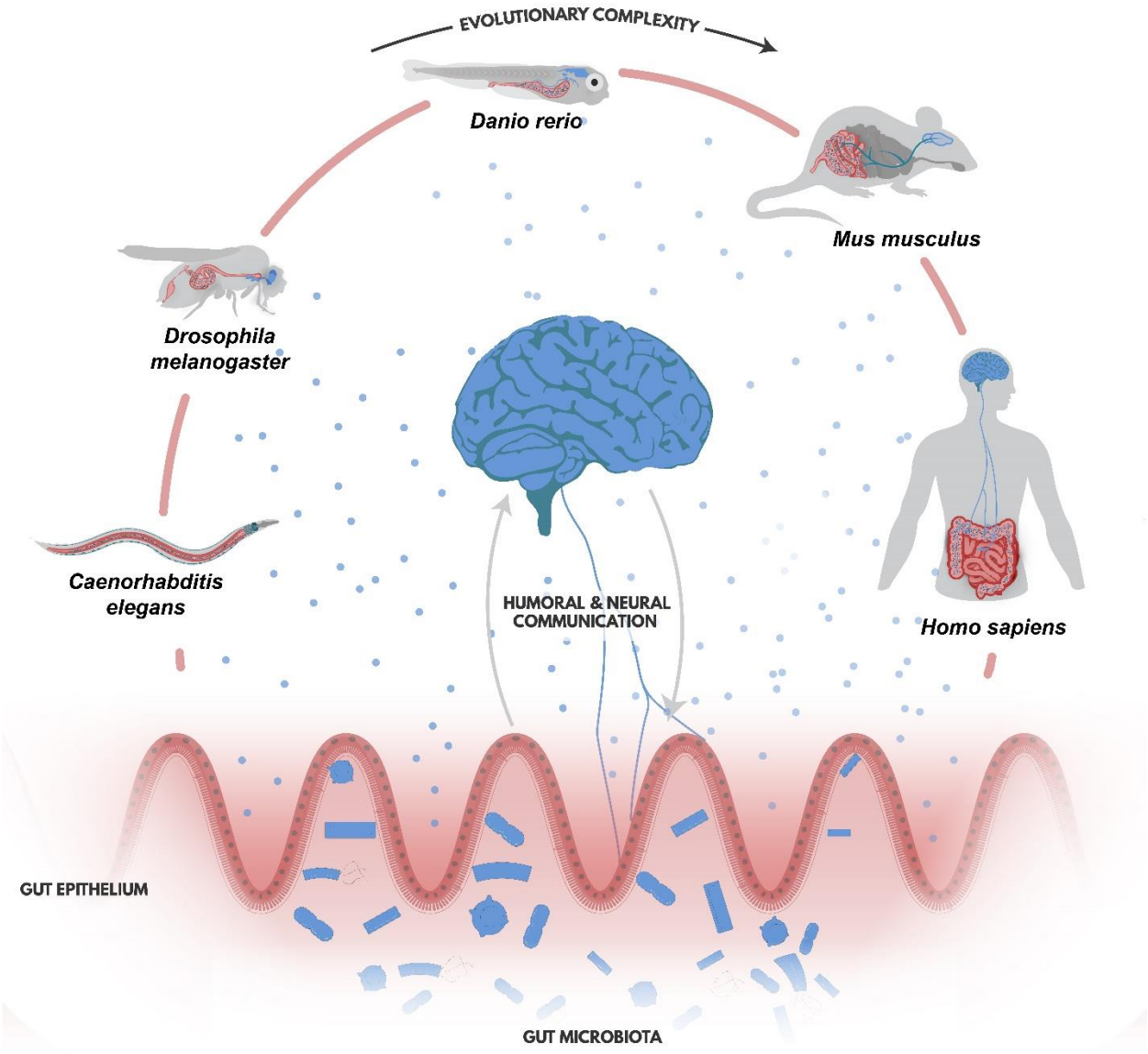

EVOLUTIONARY COMPLEXITY
Danio rerio
Mus musculus
Drosophila melanogaster
Caenorhabditis elegans
Homo sapiens
HUMORAL & NEURAL COMMUNICATION
GUT EPITHELIUM
GUT MICROBIOTA

Figure 2

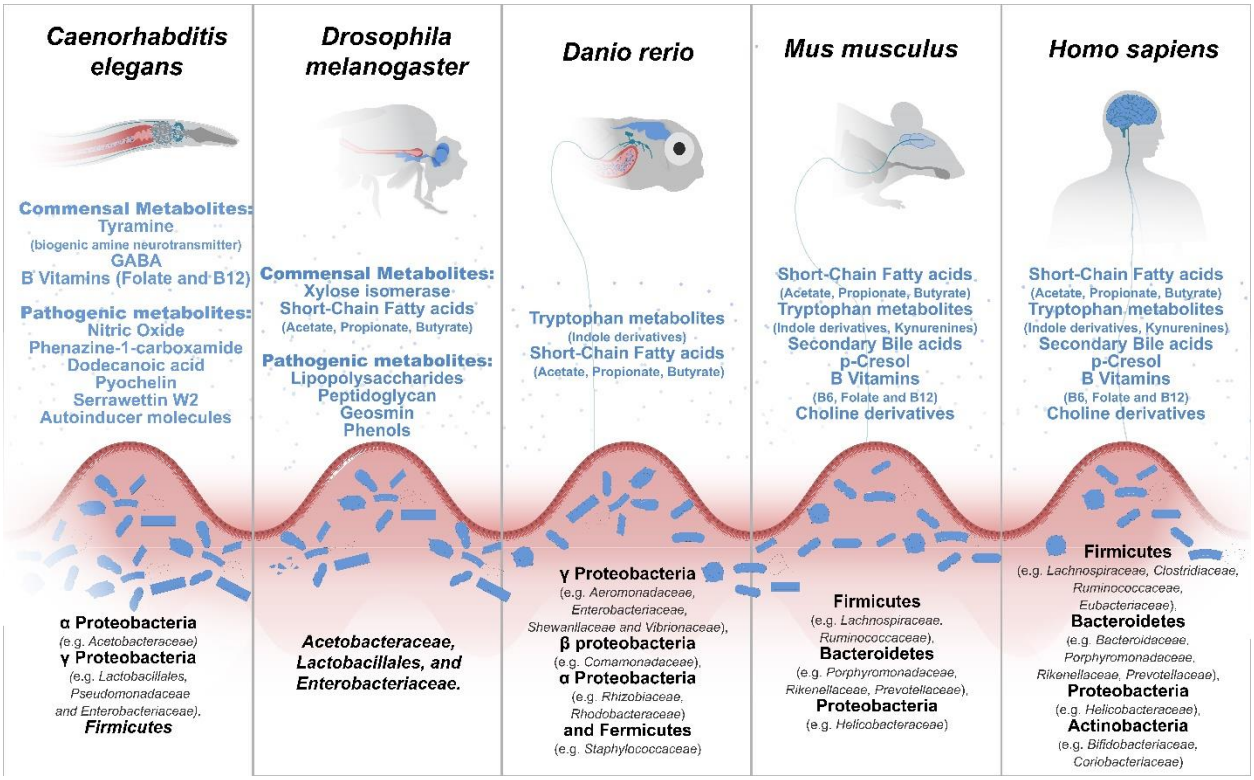

Caenorhabditis elegans
Commensal Metabolites:
Tyramine
(biogenic amine neurotransmitter)
GABA
B Vitamins (Folate and B12)
Pathogenic metabolites:
Nitric Oxide
Phenazine-1-carboxamide
Dodecanoic acid
Pyochelin
Serrawettin W2
Autoinducer molecules
α Proteobacteria
(e.g. Acetobacteraceae)
γ Proteobacteria
(e.g. Lactobacillales, Pseudomonadaceae and Enterobacteriaceae),
Firmicutes
Drosophila melanogaster
Commensal Metabolites:
Xylose isomerase
Short-Chain Fatty acids
(Acetate, Propionate, Butyrate)
Pathogenic metabolites:
Lipopolysaccharides
Peptidoglycan
Geosmin
Phenols
Acetobacteraceae, Lactobacillales, and Enterobacteriaceae.
Danio rerio
Tryptophan metabolites
(Indole derivatives)
Short-Chain Fatty acids
(Acetate, Propionate, Butyrate)
γ Proteobacteria
(e.g. Aeromonadaceae, Enterobacteriaceae, Shewanllaceae and Vibrionaceae),
β proteobacteria
(e.g. Comamonadaceae),
α Proteobacteria
(e.g. Rhizobiaceae, Rhodobacteraceae)
and Fermicutes
(e.g. Staphylococcaceae)
Mus musculus
Short-Chain Fatty acids
(Acetate, Propionate, Butyrate)
Tryptophan metabolites
(Indole derivatives, Kynurenines)
Secondary Bile acids
p-Cresol
B Vitamins
(B6, Folate and B12)
Choline derivatives
Firmicutes
(e.g. Lachnospiraceae, Ruminococcaceae),
Bacteroidetes
(e.g. Porphyromonadaceae, Rikenellaceae, Prevotellaceae),
Proteobacteria
(e.g. Helicobacteraceae)
Homo sapiens
Short-Chain Fatty acids
(Acetate, Propionate, Butyrate)
Tryptophan metabolites
(Indole derivatives, Kynurenines)
Secondary Bile acids
p-Cresol
B Vitamins
(B6, Folate and B12)
Choline derivatives
Firmicutes
(e.g. Lachnospiraceae, Clostridiaceae, Ruminococcaceae, Eubacteriaceae),
Bacteroidetes
(e.g. Bacteroidaceae, Porphyromonadaceae, Rikenellaceae, Prevotellaceae),
Proteobacteria
(e.g. Helicobacteraceae),
Actinobacteria
(e.g. Bifidobacteriaceae, Coriobacteriaceae)

Figure 3

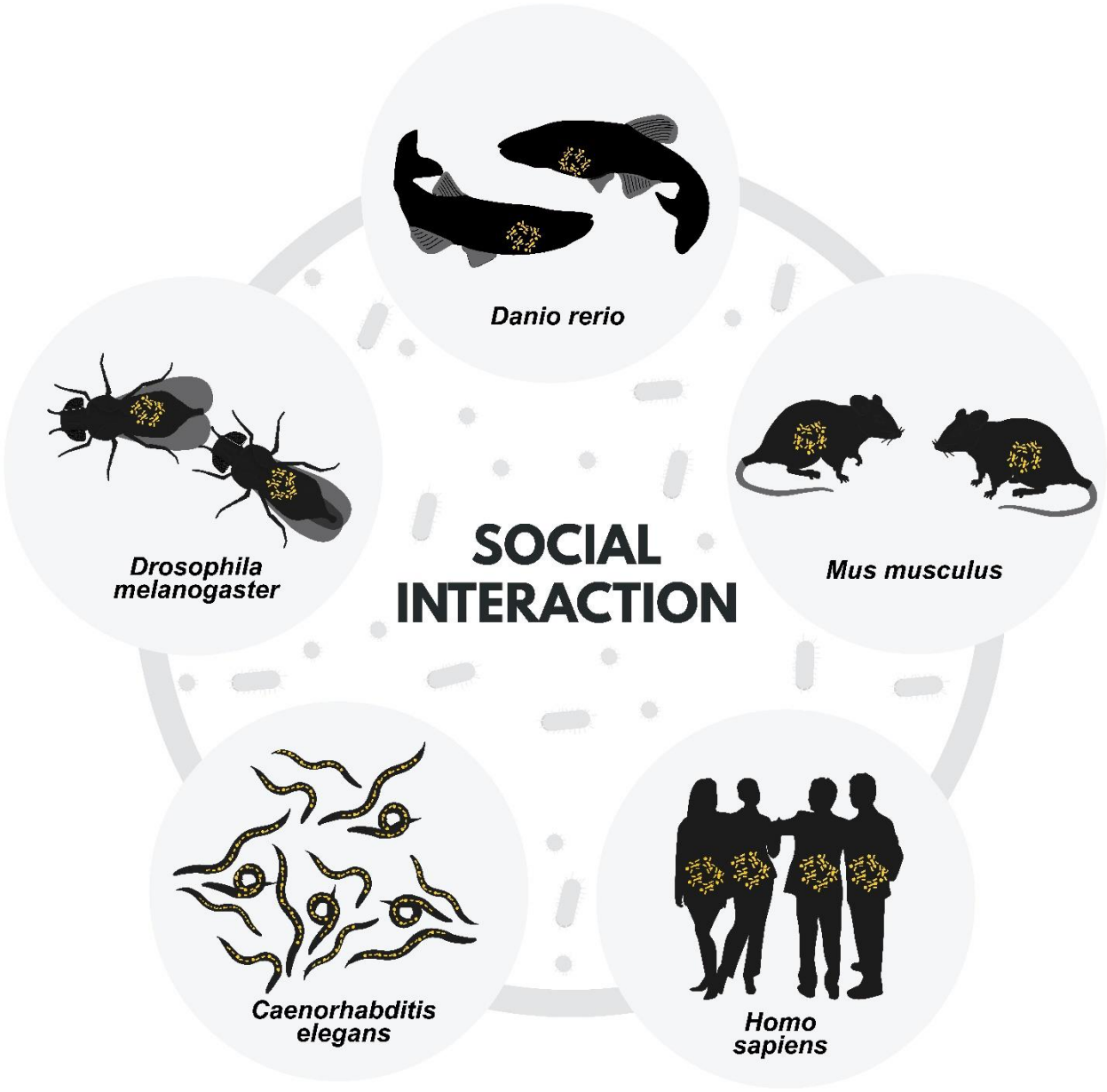
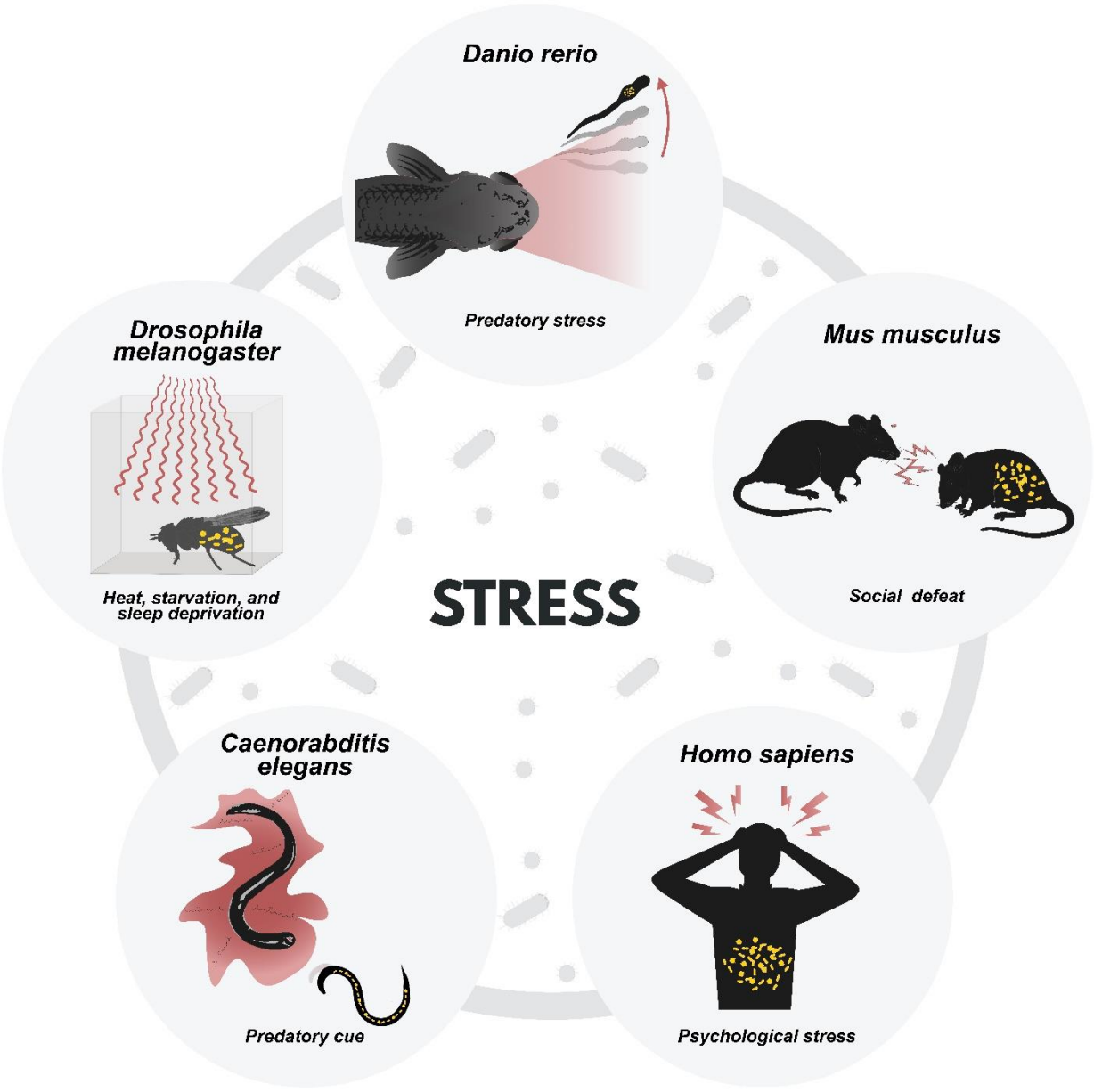

Danio rerio
Drosophila melanogaster
Mus musculus
SOCIAL INTERACTION
Caenorhabditis elegans
Homo sapiens

Figure 4


Danio rerio
Predatory stress
Drosophila melanogaster
Heat, starvation, and sleep deprivation
Mus musculus
Social defeat
STRESS
Caenorabditis elegans
Predatory cue
Homo sapiens
Psychological stress