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The diet-microbiome tango: how nutrients lead the gut brain axis

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Nutrients and the microbiome have a profound impact on the brain by influencing its development and function in health and disease. The mechanisms by which they shape brain function have only started to be uncovered. Here we propose that the interaction of diet with the microbiome is at the core of most mechanisms by which gut microbes affect host brain function. The microbiome acts on the host by altering the nutrients in the diet and by using them as precursors for synthetizing psychoactive metabolites. Diet is also a major modulator of gut microbiome composition making this another key mechanism by which they affect the host brain. Nutrient-microbiome-host interactions therefore provide an overarching framework to understand the function of the gut-brain axis.

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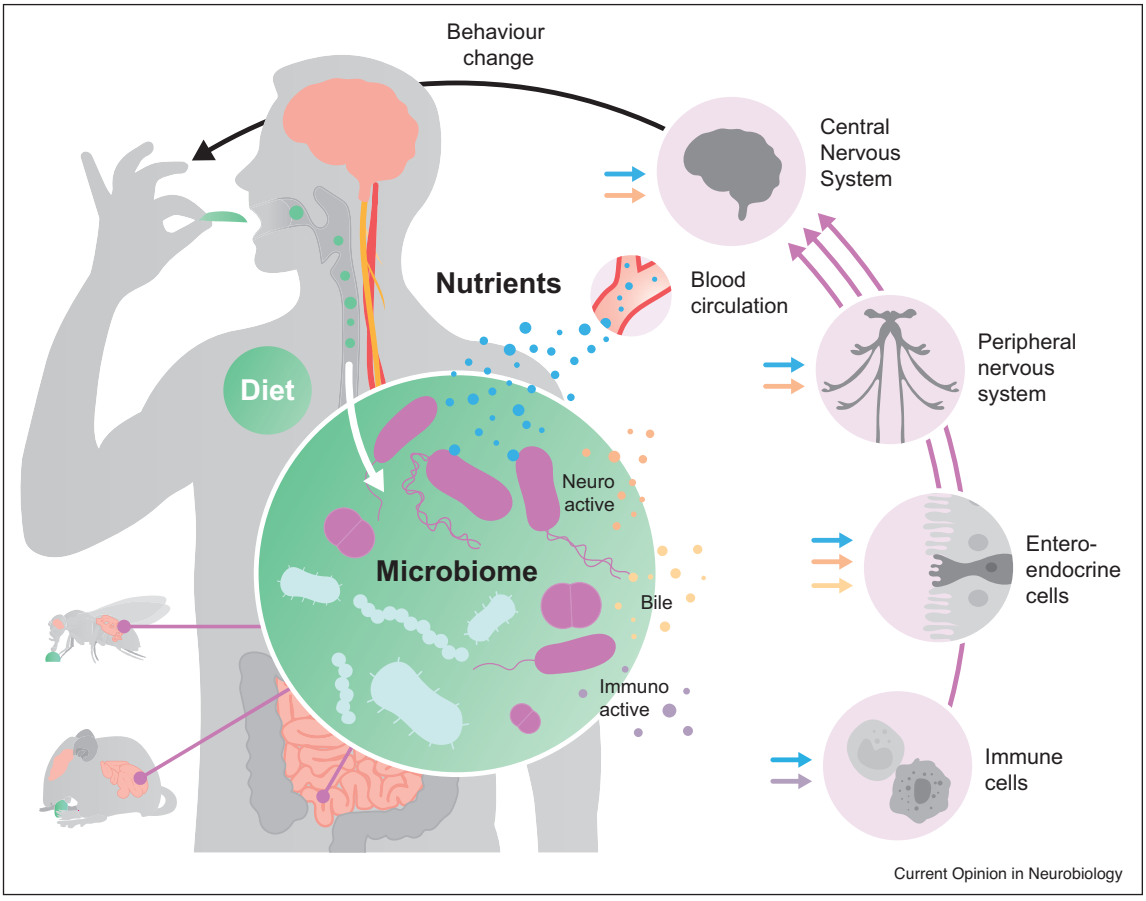
Introduction

Multicellular organisms have long been known to live in a community with microorganisms [1]. Many of these microbiota have co-evolved a mutualistic or commensal relationships with their host [2]. These relationships manifest themselves in microbiota having a strong influence on the host, ranging from physiology and metabolism, to growth and immune system function [3]. Most remarkable has been the finding that the microbiome can profoundly affect brain function and behavior [4••,5]. These effects are not confined to a specific class of behaviors but the protection from deleterious effects of stroke [6], to anxiety [7–9], social behaviors [10,11], exploration [9,12], and decision making [13••]. While in humans almost all of these influences are inferred by correlating the microbial composition of people with specific physiological or pathological conditions, gnotobiotic animal models have proven to be essential to create a causal framework to understand the contribution of specific microbes to specific phenotypes and the molecular and cellular mechanisms by which they do so [4••,14–17]. In almost all cases we are nevertheless still far from inferring a true causal relationship, as doing so requires elaborate experimental designs and the community valuing negative results [18••]. Research on how microbes influence animal phenotypes goes however far beyond its use as an experimentally tractable platform to study the human microbiome and has led to seminal insights into the symbiotic relationships between microorganisms and their host [19–21].

While a major effort in the field is still dedicated to identifying the host traits shaped by the microbiome as well as the microbes which shape these phenotypes, progress in this field requires identifying the cellular and molecular mechanisms by which commensal bacteria act on the host. This begs an essential question: Why does the microbiome play such an important role in determining host phenotypes? And is there an overarching theme providing a generalizable explanatory framework for the impact of the microbiome on the host? Here we propose that the interaction of the microbiome, and especially the gut microbiome, with host nutrition provides such an explanatory framework (Figure 1). We will use this framework to discuss possible mechanisms by which the gut microbiome could shape one of the most intriguing host phenotypes, brain function and behavior. Furthermore, we will discuss how the careful design of experiments, in which diet and microbiome are precisely manipulated is a key prerequisite to uncover mechanisms underlying microbiome host interactions.

A key aspect of this proposal is that the gut microbiome critically depends on the diet of the host for both its own growth and its biosynthetic capacities [22,23]. High fat diet (HFD) or high fiber diets, for example, are two dietary conditions which have been shown to affect microbial composition [24–26]. Furthermore, the gut microbiome relies on the host diet content to produce neuromodulators such as γ-aminobutyric acid (GABA) [27••], provides nutrients to the host, and modifies the availability of specific nutrients derived from the diet, thereby deeply shaping the nutritional environment of

Figure 1



Diet-microbiome-host interactions are the central mechanism, by which the gut brain axis influences behavior.
In most species different dietary compositions (green) change the microbiome composition by creating an environment in the gut more suitable for some bacteria than others. The gut microbiome uses nutrients from the diet to proliferate and to produce various metabolites that can influence the host, including neuroactive metabolites (orange), bile acids (yellow), immune-active metabolites (purple), and nutrients (blue). The nutrients synthetized by the microbiome (such as vitamins, amino acids and short chain fatty acids) can themselves affect brain function and behavior. These effects are conveyed via the peripheral nervous system, enteroendocrine cells, and immune cells that signal to the central nervous system (CNS), or by acting directly on the CNS to alter behavior.

the host. Two prominent mechanisms mediating this phenomenon are for example the gut bacteria changing the ability of the host to absorb specific nutrients from its diet [26,28•,29] or the biosynthesis of nutrients which cannot be synthetized by the host and are therefore provided by the bacteria without originally being in the ingested diet [30••,31••]. Given its importance one would therefore expect that diet-microbiome interactions must be a central factor driving the evolution of microbiome host interactions including its effect on brain function and behavior. Diet should therefore be given special consideration when studying the mechanistic basis of how the microbiome acts on the brain and is very likely to be an important part of such mechanisms.

This idea is supported by findings that the nutritional state of the animal profoundly shapes behavior by, for example, altering reward mechanisms and higher cognitive functions such as risk assessment, as well as social behavior [32,33,34•]. A further way of conceptualizing the effect of diet on the brain is to think of nutrients as acting on the host in a similar way to hormones [35]. Hence our suggestion that microbiome interactions with host diet provide an important explanatory framework to understand the microbiome effect on host brain function and behavior (Figure 1).

The microbiome shapes the nutritional environment of the host

An important mechanism by which the gut microbiome could act on behavior is by altering the availability of nutrients to the host (Figure 1). Experiments based on gnotobiotic animal models and metabolite profiling of isotopically labelled nutrients have shown that various

bacterial strains can produce important nutrients for the host. But the bacterial community can also compete for nutrients from the host diet or metabolize those that are unusable by the host thereby making them available. By producing, removing or altering nutrients in the diet, gut bacteria can profoundly alter the nutrient availability to the host and hence the impact of the diet on brain function.

Amino acids

A class of essential nutrients that are provided by gut microbiota are amino acids (AAs). AAs can significantly influence host behavior. They can act themselves as important neurotransmitters (such as L-Glutamate) or be the precursors for the synthesis of important neurochemicals such as serotonin, γ-aminobutyric acid (GABA), dopamine or norepinephrine. Importantly, gut microbiota, including *Bifidobacterium* and *Lactobacililus* strains, can metabolize dietary AAs in their own capacity to produce these psychoactive compounds [36]. It is however important to note that it still unclear to what extent microbially produced neuromodulators can act beyond the intestinal tract to directly influence brain function.

The lack of a single essential AA in the diet can also clearly affect metabolism and feeding related behaviors, including exploration-exploitation tradeoff calculations by signaling the nutrient state of the animal [13••,32,33,37,38]. Neuronal AA availability also impacts mental health. The neuronal lack of AAs profoundly alters neuronal gene transcription and in humans and rodent models leads to pathological states with many hallmarks of neurodevelopmental disorders [39–41]. Furthermore, in early experiments where human subjects where fed diets lacking single essential AAs the subjects reported increasing symptoms of exhaustion, nervousness and dizziness [40]. While it is clear that AAs affect brain activity, the molecular mechanisms by which AAs are sensed by the brain to alter behavior are still mostly unknown, but are likely to rely on specific nutrient sensing mechanisms [33].

Gut microbes have been shown to alter AA availability to the host in two ways: One is by increasing the digestion of proteins and the subsequent absorption of AAs or by synthetizing and providing AAs to the host. In *Drosophila melanogaster*, gut microbiota can increase the AA influx to the host [28•,42]. One of the mechanisms by which this is achieved relies on the commensal *Lactobacillus plantarum* altering immune signaling in the gut, leading to an increase in the expression of gut peptidase encoding genes [28•,43]. This results in an increase in the proteolysis of dietary proteins and the absorption of AAs.

The other mechanism by which gut microbes can change AA availability is by directly synthetizing and providing AAs, including essential ones, to the host. The classic examples for this important mechanism are aphids in which most essential AAs are provided by *Buchnera*, their obligate bacterial symbiont [19] and ruminants, in which gut microbes provide a large fraction of circulating AAs [44]. In humans, microorganisms in the intestinal microbiome can produce considerable amounts of AAs (1–20% of the total pool) [30••]. This is a nice example, in which the microbiome expands the biosynthetic capacity of the host, allowing it to have access to metabolites not present in the diet and whose biosynthetic capacity is not encoded in the host genome.

The microbiome can also compete for essential nutrients, such as AAs, in the diet. For instances, some gut microbial species metabolize choline, an essential nutrient for the human host with a metabolism related to AAs, into trimethylamine (TMA). In gnotobiotic mice colonized with TMA-producing bacteria, such as Desulfovibrio desulfuricans, the bioavailability of choline to the host is remarkably reduced compared to animals colonized with non-TMA-producing bacteria [29].

In conclusion AAs play a central role in modulating brain function by acting as precursors for important neurochemicals and by signaling the nutrient state of the animal. The microbiome is an important modifier of AAs availability to the host and uses these to synthetize many important metabolites acting on neurons. AA-microbiome-host interactions are therefore an important mechanism by which the microbiome acts on the brain.

Digestible and non-digestible carbohydrates

Though dietary carbohydrates are not considered to be essential for humans [45], the balance between simple sugars and non-digestible carbohydrates (such as dietary fibers) seems to play an important role in health, mental capacity and development as well as behavior [46–50]. Glucose availability is directly sensed by neurons in the central nervous system (CNS) and has a direct effect on neuronal activity [51]. Insufficient amounts of glucose have been associated with mental retardation during development, and with reduced mental capacity and self-control in adulthood [46–49]. Alzheimer disease onset is also associated with reduction in cerebral glucose metabolism [48]. Diets containing different levels of carbohydrates can clearly affect not only metabolism and energy expenditure, but also feeding drive, exploration, and locomotion [52–54]. Finally, a chronic over ingestion of sugars has been linked to changes in brain function including impairments in chemosensation [55], learning and memory and in reward related behaviors in rodents [56] and have been associated with pathological changes in the reward circuitry of humans [57,58].

The gut microbiome modulates the energy derived by the host from digestible and non-digestible carbohydrates.

One of the key mechanisms by which the gut microbiome is thought to act on the host relies on its ability to consume both of these carbon sources and to produce short-chain fatty acids (SCFAs) as well as other metabolites from it [59]. Depending on microbial composition, isocaloric diets differing in their carbohydrate composition can, therefore, represent different metabolic scenarios for the host. Although SCFAs are typically thought of as metabolites directly acting on the brain, their effect as a caloric nutrient should not be disregarded. In humans SCFAs generated from dietary fibers are known to contribute approximately 10% of the human caloric requirement [60]. Therefore when the dietary ratio of non-digestible fibers is high the effect of bacteria such as *Bacteroides thetaiotaomicron* can be especially profound as it significantly increases the amount of energy harvested form diet [61]. Hence, pushed to the extreme, when exposed to diets that include fibers, the microbiome can supply the host with energy harvested from the diet, while in diets rich in simple sugars, microbiota can function as competitors for that energy source. A clear example for such a competitive effect on behavior was observed by exploring the effect of the gut microbiome on food choice behavior in flies using a chemically defined (holidic) diet [13••]. Flies inoculated with 2 bacterial strains (*Lactobacillus plantarum*^{WJL}, *Acetobacter pomorum*), have a drastically increased appetite for sugar when compared to germ-free controls, which is reverted by adding increasing amounts of sugar to the diet. These findings are in agreement with other studies showing that bacteria deplete sugars from the fly diet when these are only available in limiting amounts [62]. Flies with a microbiome are therefore likely to be sugar deprived, making them very sensitive to the sugar content of the diet and hence to the many effects of sugar deprivation including alterations in locomotion and other behaviors modulated by the motivational state of the animal [12]. This example also nicely exemplifies the importance of controlling and precisely varying the nutritional content of the diet when studying the impact of the microbiome on behavior, as such effects might only be observed under specific nutritional conditions.

Vitamins

The microbiome has been shown to be the source of vitamins for a variety of hosts, ranging from invertebrates to humans [63,64]. Importantly, lack of these vitamins can influence the CNS directly, as they are implicated in many neurological functions, and actively cross the blood brain barrier via dedicated transporters [65]. Vitamin B_9 (folate) has a critical role for prenatal neurodevelopment, as well as implication in modulating depression, [65,66]. In *Drosophila* thiamine (Vitamin B_1) is essential for its full development into adulthood [31••]. Germ-free flies reared in the absence of this vitamin do not pupariate. However, when associated with *Acetobacter* stains that synthetize this vitamin, flies develop normally in thiamine-free medium, illustrating the ability of particular bacterial strains within the microbiome to strongly impact the health of the host by providing a vital nutrient. When testing the source of a lack of phenotype for a dietary deficiency it is thus essential to consider the potential of the microbiome to supply nutrients, such as vitamins, to the host as strongly as the possibility that novel biosynthetic pathways are activated in the host in such extreme cases [67]. Also in humans, the metabolic pathways for the synthesis of Vitamin C and of several vitamins from the B complex, such as, biotin, riboflavin, pantothenate, folate, and thiamine, are fully represented in the metagenome of the human microbiome, which highlights its potential to act as an important source of these nutrients to the human host and hence neurodevelopment and brain function [68].

Fatty acids

A sufficient intake of different essential (Omega-3 and Omega-6) and non-essential fatty acids is also pivotal for health as shortage of these compounds has detrimental effects on brain function and behavior [69]. Dysregulation of long chain fatty acids (by fatty acid synthase) affects both sleep and metabolism [70], while deficiencies in Omega-3 are correlated with increased aggressive behavior in humans [71] and severely affect learning and memory in bees [72•]. Importantly, some microbial species, such as *Prevotella*, *Lactobacillus*, and *Alistipes* are likely to increase the levels of saturated long-chain fatty acids (SLCFAs), such as heptadecanoic (C17:0) and stearic acids (C18:0) in rats [73].However a clear link between microbiome induced alterations in fatty acids and brain function remains to be shown.

In conclusion the gut microbiome, can shape the nutritional "reality" of the host depending on its microbial composition and on the nutrients available in the host diet. This supports the idea that in many cases the microbiome exerts its profound behavioral effect by altering the nutrient availability to the host. The microbiome can either buffer nutrient scarcity as in the case of vitamins and AAs, "help" the host to utilize macronutrients such as fibers, or compete with the host for them, thus ultimately altering CNS and peripheral nervous system nutrient availability for the host (Figure 1). Therefore, given that functions of nutrients on the brain are mediated by specific neuronal nutrient sensing mechanisms [33], these and the circuits in which they act are attractive candidates for mediating effects of the microbiome on the host.

Diet shapes microbiome composition and hence its impact on host brain function

Diet shapes microbiome composition

As discussed above microbes can alter the nutritional content of the host diet and thereby exert important effects on brain function. But it is also well established that changes in diet composition lead to a rapid adjustment in the relative abundance of microorganisms in the

microbiome [74,75•,76••,77••,78]. This is a topic on which a large amount of work has been done in humans. Several specific characteristics on the diet have been found to have a strong association with changes in gut microbial communities. For example, Indian populations consuming different diets show corresponding separate microbiome compositions [76••]. More specifically, the microbiome of subjects from North-Central India, which consume primarily a plant-based diet, was associated with an increased *Bacteroidetes* to *Firmicutes* ratio, and over representation of the pathways for branched chain AA (BCAA) biosynthesis and degradation of complex polysaccharides. This is likely due to the low abundance of BCAAs and high abundance of complex carbohydrates in this diet. While, the microbiome of subjects from Southern India, consuming mainly an omnivore diet (including significant amount of animal protein), was associated with increased abundance of functional categories related to BCAA transporters, reflecting the higher availability of these AAs in the diet [76••].

A key modulator of microbiome composition and diversity is dietary fiber [25]. Dietary fibers are thought to promote the growth of specific bacteria, and decrease, for example, the *Firmicutes* to *Bacteroidetes* ratio [79••]. Macronutrients, including carbohydrates, fats, and proteins seem to also affect the integrity of the microbiome [22]. Sustained exposure to HFD, for example, is thought to increase the *Firmicutes-to-Bacteroidetes* ratio [79••,80]. Other dietary components, such as proteins, probiotics and simple carbohydrates have also been shown to affect microbiome composition [81].

The changes in microbiome composition however show a stronger association with specific aspects of food composition rather than with the macronutrient composition of meals [77••]. Meaning that the combination of nutrients and the context of the nutrient content, including fiber content or the presence of substances with antimicrobial activity such as phenolic acids, are more relevant to predict the composition of the microbiome than the global macronutrient composition of the diet [22,77••].

Not only the composition of the diet but also how it is processed has a strong impact on the microbiome. The pre-treatment of foods (e. g. cooking) increases microbe diversity, and lowers the relative proportion of *Bacteroidetes* (which can degrade glycans), in part because heat decreases the levels of compounds with antimicrobial properties [82]. This effect seems to be specific to plant-based foods, as cooked meat does not change the microbiome composition compared to ingesting raw meat. Diet has also been found to shape the virome, which in turn shapes the gut bacterial ecosystem [83,84•]. For example, SCFAs and fructose trigger the production of bacteriophages in *Lactobacillus reuteri* residing in the gut of mice [84•]. This decreases the abundance of this bacterium in response to the host dietary intake, highlighting that phage production can be an important mechanism through which diet changes the abundance of gut microorganisms.

Additionally, the impact of diet on host physiology is known to be highly personalized [85••]. Interestingly, the changes in microbiota composition triggered by different diets are also very personalized, while being highly reproducible within individuals [75•,77••]. This indicates that although microbial communities can be affected differently between individuals, subjects have a strong tendency to host specific bacterial compositions when exposed to a given diet, and these will reliably change with a different diet. Together with the personalized impact of diet on physiology the personalized aspect of diet-microbiome-host interactions reinforces the importance of tailoring dietary and microbiome interventions to the specific profile of the individual being treated.

Finally, the discovery that the microbiome can profoundly alter the dietary choices of the host adds an additional dimension to these complex interactions [13••]. This enforces the idea that, as in other ecological multi-species settings, microbiome-host interactions have to be understood as complex webs and not simple linear pathways.

Microbiome composition can shape the effect of diet on the host

The impact of diet on the microbiome composition is emerging as a powerful explanatory framework for understanding how diet can impact the host. A striking recent example is the discovery that the anti-seizure effect of the ketogenic diet, composed of animal and plant-based fats, proteins and fibers but no absorbable carbohydrates, can be partially attributed to changes induced in the microbiome [86••]. Mice maintained on a ketogenic diet show an increase in *Akkermansia* and *Parabacteroides* which can protect the animal from seizures by modulating host AA metabolism and altering the levels of important neurochemicals in the brain. Interestingly, this effect seems to rely on the ability of these two bacteria to form a syntrophic community.

The 'Mediterranean diet', abundant in fatty acids, vitamins, and dietary fibers, has been suggested to reduce depressive symptoms [87–89]. Similar to the ketogenic diet, the effect of the 'Mediterranean diet' on the host has been suggested to be mediated by the anti-inflammatory effect of gut bacteria [90]. However, to what extent the bacterial composition really plays a role in an 'anti-depressive' effect of the Mediterranean diet remains to be shown.

Bacterial community effects are very important to understand the impact of the microbiome on the host. When

different bacterial species enter a metabolic relationship in which they cross-feed each other they can grow in specific diets even if these would be deleterious to single members of the community [91]. Furthermore, when present in such communities, microorganisms produce metabolites which they would not be able to produce on their own. In *Drosophila* adults, *A. pomorum* and *L. plantarum* form a syntrophic community which profoundly alters the food preferences of the fly [13••,92•]. *Lactobacillus* provides lactate to *Acetobacter* which uses it to synthetize AAs which are then used by *Lactobacillus* to grow on diets lacking essential AAs. Importantly, lactate is also used by *Acetobacter* to change protein appetite in flies. Interestingly, AA production by *Acetobacter* is unlikely to account for the changes in protein appetite. Most likely *Acetobacter* uses lactate to synthetize metabolites which then act to change food preference. The emerging picture is that bacterial communities in the gut of the host are able to create metabolic conditions which allow them to overcome unsuitable dietary conditions, allowing them to exert a strong effect on host behavior.

The effect of diet-microbiome interactions on behavior relies on the action of nutrients or diet derived metabolites on different host cell types and organ systems

A large body of work has shown that specific host behavioral traits are associated with specific microbiome compositions. Multiple studies in recent years have gone beyond these correlations and have shown the underlying causal mechanisms. As discussed above many of these mechanisms rely on diet-microbiome interactions and in many of these cases the communication between the microbiome and the host is primarily occurring via nutrients and metabolites secreted by the bacteria [4••]. These metabolites can be well known psychoactive metabolites (i.e. neurotransmitters or agonist or antagonist of their receptors), immune activating metabolites (e.g. lipopolysaccahrides; LPS), nutrients (vitamins, SCFA, AAs), and other metabolites such as bile acids [23].

These metabolites can be detected within the gut by enteroendocrine cells (EEC) or the enteric nervous system (e.g. vagal nerve), or they can act at a distance and directly reach the brain via the circulatory system [4••,93,94]. Importantly, some of these have also been shown to act on glia which can also be a powerful way to alter brain function [95•]. Bacterial metabolites can also act indirectly by triggering an immune response which then alters neuronal functions [96] or by affecting metabolic responsive organs that in turn release endocrine signaling molecules [33] (Figure 1).

An interesting example of a mechanistic dissection of how gut bacteria affect behavior is recent work showing that *L. reuteri* treatment increases social interaction time in a genetic and idiopathic mouse models of autistic spectrum disorder (ASD) [10]. Experiments manipulating the vagus nerve showed that this neuronal pathway was essential for the behavioral effect of *L. reuteri*. Likewise, SCFAs, such as butyrate and propionate, can *per se* stimulate vagal afferent neurons or the release of hormones from EECs to modulate food intake as well as anxiety-related phenotypes [97,98]. Indeed, these bacterial fermentation products have been suggested to promote several ASD behavioral phenotypes, including repetitive behaviors and reduce social interaction, and thus link specific 'bacterial metabolic activities to the pathology of this disorder. Although a stringent test for causality is still missing [99,100]. These examples emphasizes the intimate relation between diet, microbiome, nutrient availability, brain function and physiology.

A poorly explored avenue is the importance of nutrient sensing signaling pathways and nutrient sensing neurons in mediating the effect of the microbiome on behavior. While first steps have been made, identifying the mode of action of the microbiome and its metabolites on the brain by identifying its targets in the host remains a key challenge for the future. Obviously, the above described well-defined separate modes of action of the microbiome on the host is a simplification, as all the organs and cell types affected by microbial metabolites also affect each other. This highlights the challenges faced when dissecting physiological networks. But current advances in modern imaging approaches, functional genomics and molecular genetics approaches, including synthetic biology, combined with computational and modeling approaches make such an endeavor feasible. These are therefore exciting times to work on this topic!

The effect of microbiome on behavior should be studied in a well-defined dietary context

Knowing that the diet alters the microbiome composition, the fact that most studies on the effect of the microbiome on host traits are correlative limits their interpretation. Especially when studying pathologies which profoundly alter dietary habits of patients. It is plausible that many behavioral alterations observed in patients which also modify nutritional preferences and eating habits will lead to changes in microbiome composition. This will easily be interpreted as the altered microbiome causing to some extent the disease. It is very difficult to control for the effects of dietary changes on microbiome composition and as such this possible indirect explanation should always be taken into account. Also medication, especially psychoactive substances have been shown to alter microbiome composition, adding an extra confounding factor in such studies [101,102]. Such caveats do not preclude the importance of the microbiome as a possible modulator of human pathologies, including diseases of the mind. The interaction of the microbiome with nutrients and psychopharmaca remains an important new avenue for explaining the etiology of diseases and developing treatments.

Animal models are therefore a powerful tractable system to identify the mechanisms by which the microbiome acts on the host. Especially relevant for microbiome research is the ability to tightly control the dietary parameters of the experiment. The failure to tightly control and accurately report the dietary conditions in which the experiments are performed might lead to the failure to reproduce findings across laboratories. Often researchers also misinterpret the effect of altering-specific dietary parameters in the diet as causing the observed phenotype. A good example is the interpretation of the interaction of a putatively obesogenic microbiome with the high fat content of the diet [26,79••]. The problem arises from the need to 'compensate' for the alteration in one nutrient in the diet by altering other parameters in the diet. Sometimes different diets reported as mainly differing in one nutrient are completely different. And especially relevant for rodent microbiome research, it is often the fiber content of the diet which is altered to 'compensate' for other changes in nutrient content. While able to reproduce original findings in the literature, studies which control for such factors sometimes find that the original claim of specific microbiome nutrient interactions are not as clear as originally thought and are likely to be caused by unintended alterations in the diet [79••]. In this context the availability of chemically defined diets (holidic diets) such as the Piper media for *Drosophila* [103,104] are ideal tools to surgically dissect the interaction of diet, microbiome, and the host [13••,92•,105].

Conclusions

Nutrition is an important explanatory framework when studying the associations between microbiome and behavior. Micronutrients and macronutrients have a pivotal role in neurodevelopment, brain function, and they are involved in modulating various behavioral phenotypes. The microbiome, in contrast, is both affected by the diet, utilizes nutrients as metabolic precursors, and also alters its nutritional content, as different bacteria can synthesize or utilize nutrients in the host diet. As nutrients can not only affect metabolic aspects of the organism's function, but also activate cascades of responses that affect neuronal integrity and behavior, the diet-microbiome interactions have to take a center stage when attempting to untangle the effect of the microbiome on behavior, including when reproducing such experiments.

We propose that the impact of the microbiome on the brain and behavior is likely to have emerged from the ability of the microbiome to alter the nutritional content of the host diet and hence its nutritional 'reality'. As such diet-microbiome-host interactions remain at the core of many of the actions of the microbiome on behavior but have expanded beyond the direct alterations of the nutrient content of the diet. An interesting example might be the ability of an AA synthetizing bacterial community to alter protein appetite in the fly. While this critically depends on the AA state of the host and is mechanistically intertwined with AA metabolism in the bacterial community, the behavioral effect of the microbiome is independent on its ability to provide the host with AAs and is likely to rely on a separate mechanism [13••,92•].

Given the great variability of inter-individual microbiome composition, it is important to consider not only dietary caloric and macronutrient composition but also the metabolic potential of the individual's microbiome [106–108]. In addition, considering the strong interplay of diet and microbiome, when studying the effect of nutrition on behavior, one must aspire to test groups with (as much as possible) similar microbiome compositions.

Ultimately, studies aiming at a mechanistic understanding of how the microbiome alters brain function and behavior, should include a detailed analysis of the metabolome, ideally using stable isotope labelled precursors [18••,109], in order to better understand which metabolites and nutrients are derived from the microbiome. Moreover, such research will highly benefit from microbial genetics methods. These allow the manipulation of the ability of gut microbes to generate specific metabolites by mutating the genes encoding enzymes in pathways involved in their production [92•,110]. Such experiments will enable a detailed mechanistic understanding of how gut bacteria are modified and modify diet to alter host brain function and behavior.

Conflict of interest statement

Nothing declared.

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