OPINION

Why does the microbiome affect behaviour?

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Abstract | Growing evidence indicates that the mammalian microbiome can affect behaviour, and several symbionts even produce neurotransmitters. One common explanation for these observations is that symbionts have evolved to manipulate host behaviour for their benefit. Here, we evaluate the manipulation hypothesis by applying evolutionary theory to recent work on the gut–brain axis. Although the theory predicts manipulation by symbionts under certain conditions, these appear rarely satisfied by the genetically diverse communities of the mammalian microbiome. Specifically, any symbiont investing its resources to manipulate host behaviour is expected to be outcompeted within the microbiome by strains that do not manipulate and redirect their resources into growth and survival. Moreover, current data provide no clear evidence for manipulation. Instead, we show how behavioural effects can readily arise as a by-product of natural selection on microorganisms to grow within the host and natural selection on hosts to depend upon their symbionts. We argue that understanding why the microbiome influences behaviour requires a focus on microbial ecology and local effects within the host.

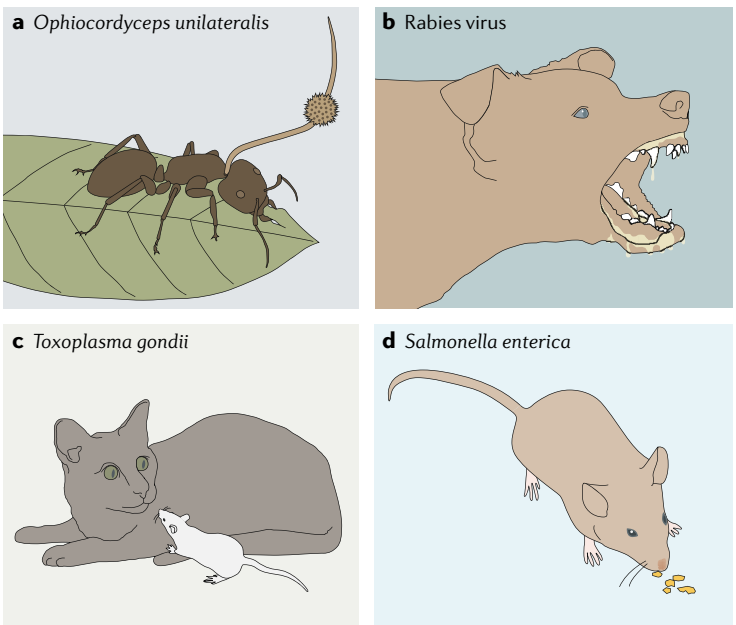
The link between the gut and brain has been discussed for centuries, with multiple proposed mechanisms underlying this relationship[1]. These include communication through the vagus nerve[2], the immune[3] and endocrine systems[4] and microorganism-derived neuroactive chemicals[5]. The relative importance of these routes and how they might interact are unclear, but studies are increasingly documenting effects of gut microorganisms on the brain and behaviour[6–9]. To describe these relationships, the term 'microbiota–gut–brain axis' has been coined[10]. For example, faecal microbiota transplantation in mice can cause the behavioural traits of the recipient to become more like those of the donor[11]. Behavioural effects have also been traced to specific subsets of the microbiota. Evidence suggests that *Lactobacillus* and *Bifidobacterium* species can alleviate anxiety and depressive-like symptoms[12–17], including in humans[18,19]. Particular *Lactobacillus* species can also improve social interactions in stressed mice[20] and restore impaired oxytocin production and social deficits driven by a high-fat maternal diet[21]. In addition, *Bacteroides* species have been shown to ameliorate repetitive and anxiety-like behaviours and communicative impairments in mice, seemingly through restoration of a specific bacterial metabolite[22].

Such microbiota-driven alterations of host behaviour have led to the hypothesis that some symbionts manipulate the host for their own ends[23–26]. For example, microorganisms might make us more sociable[27], and even altruistic[28], in order to increase host contacts and enhance their transmission. The general idea of behavioural manipulation — whereby a microorganism evolves to change host behaviour because this increases microbial fitness[29] (for example, promoting its own transmission) — has its roots in parasitology[30]. Numerous parasites affect the host nervous system and drive atypical behaviour (BOX 1), often by interfering with neurotransmitter or neuropeptide signalling[31,32]. These effects are commonly attributed to manipulation by the parasite[30]. Many examples come from invertebrate hosts, and one particularly striking example is the fungus *Ophiocordyceps unilateralis*, which infects insects, including ants. In this example, two key pieces of evidence support evolved parasite manipulation. First, infection with the fungus induces ant hosts to adopt a certain elevation in the canopy and then bite on vegetation, anchoring the ant before fungal sporulation[33,34]. Second, and critically, there is evidence that this provides fitness benefits to the parasite, as the particular elevation (and likely humidity) that the ant adopts appears important for fungal development[33]. In mammals, examples of parasites that affect the behaviour of their hosts are rare but include rabies virus and *Toxoplasma gondii*[35–37]. However, although evidence indicates that parasites can influence host behaviour, the second step — that the parasite gains a fitness benefit from its effects — is difficult to demonstrate. This difficulty is because it is typically challenging to show that the change in host behaviour resulting from parasite infection increases the fitness of the parasite in its natural environment. Thus, even in the parasite field, it is unclear whether some long-discussed examples of parasite manipulation are genuine[38].

The parasite literature, therefore, teaches us that demonstrating evolved manipulation is experimentally challenging. This literature also makes evolutionary predictions about when one should expect host manipulation. Here, we apply the evolutionary theory of parasite manipulation[39,40] and host–symbiont interactions[41] (BOX 2) to the mammalian microbiome (FIG. 1). We consider the possible routes by which natural selection may have led to host manipulation by gut microorganisms and conclude that manipulation of host behaviour is often unlikely. We explore other evolutionary explanations for the behavioural effects of mammalian symbionts and propose that they modulate behaviour as a side effect of natural selection on other functions. In particular, host-affecting compounds can arise as a by-product of natural selection on microorganisms to compete within or control the local environment. Finally, hosts may evolve to

Box 1 | **Examples of parasites affecting host behaviour**

The fungal parasite *Ophiocordyceps unilateralis* induces ants to reach a certain elevation in the canopy, where they then bite on vegetation with the so-called death grip[33] (see the figure, part **a**), thus securing a position in the canopy that is favourable for fungal growth. The fungus then emerges from the base of the ant's head to sporulate[33]. In vertebrates, parasite infections can change the social behaviour of hosts in ways that may promote parasite transmission[35]. For example, rabies virus infects mammals, including dogs and humans. The virus causes inflammation of the central nervous system and increased host aggression (see the figure, part **b**), which leads to biting and transmission[35]. The protozoan parasite *Toxoplasma gondii* infects birds and mammals and has been shown to reduce the aversion of rodents to cat urine[36,37] (see the figure, part **c**). This may put the rodent at greater risk of predation and increase the chance of parasite transmission to feline hosts, which is necessary for the parasite to reproduce sexually[36]. Infection can cause sickness behaviour in hosts, including behaviours such as appetite loss[53]. The evolutionary basis for sickness behaviour is not always clear, but loss of appetite may have evolved to decrease nutrient supply to intestinal pathogens. Interestingly, there is evidence that *Salmonella enterica* subsp. *enterica* serovar Typhimurium suppresses this appetite loss (see the figure, part **d**), which may represent manipulation of host feeding behaviour[52].



depend on microbial metabolites for normal physiological function; consequently, if the microorganism that produces these metabolites is lacking, then behavioural dysfunction can result.

Manipulation of host behaviour

The potential benefits to a symbiont from manipulating host behaviour, which we define here as global manipulation (FIG. 1), are clear; how a host behaves can strongly affect the growth and survival of a symbiont and its transmission to other hosts. Despite this, the conditions that favour the evolution of a manipulative trait are quite restrictive[39,40] (TABLE 1). Consider a bacterial strain that uses a dedicated set of enzymes to generate a compound that affects host behaviour. Moreover, let us assume that this compound influences host behaviour in a way that benefits the bacterium. Hypothetically, it could immediately make the host more sociable and increase the potential routes of transmission to new hosts, or it might make a host consume resources that the bacterium needs. When will the production of this compound be favoured by natural selection? If the host is colonized only by this single strain, then production of the compound is predicted to evolve so long as any fitness cost of production is outweighed by the benefits of increased nutrients or transmission.

If the bacterium must compete for resources and space with other strains and species, however, the prediction is very different. Whereas the metabolic cost of the enzymes falls on the producing bacterium, the benefits are now shared by multiple members of the microbiota. Indeed, in the case of a transmission effect, it is likely that much of the microbiota benefits. If a bacterial strain manipulates host food preference, then only strains in a niche similar to that of the producing strain may benefit, but these are also the main competitors of this strain. In either of these cases of hypothetical manipulation, therefore, the prediction is that a strain in the same niche that lacks the enzymes will outcompete the producing strain because it receives the benefit without paying the cost. This competitor could be a loss-of-function mutant of the producing strain or another species that inhabits the same niche. Ultimately, this is predicted to lead to the loss of the manipulating compound[39,40]. For high costs of production, this loss is expected to happen rapidly, in a few microbial generations. For low costs, the loss is predicted to take longer. Low costs are possible because natural selection is expected to minimize the costs associated with a given trait for a given level of benefit[42]. Consistent with this concept, some parasites of invertebrates are thought to act by increasing synthesis of neuromodulators, such as serotonin, by the host, which may be less costly than producing them themselves[31,43]. A low cost may also be facilitated when a microorganism can use pre-existing metabolic pathways to drive host effects. However, even for low metabolic costs, the prediction is that a manipulative trait will eventually be lost in the face of prolonged competition from a non-producing strain within a host.

Evolutionary theory then predicts that the evolution of manipulation will critically depend on the diversity within the microbiota and, more specifically, how much competition a given strain experiences with other genotypes in its niche. If a strain is largely free from such competition, then manipulation is predicted to evolve if affecting host behaviour can increase resources or transmission. However, when a strain faces competition from other genotypes, the evolution of costly mechanisms of manipulation is disfavoured, as these will undermine the ability of a strain to persist in the microbiota. The question then is which of these two scenarios best represents a given host microbiome. The human gut is an ecologically complex community, estimated to contain hundreds to thousands of interacting species and strains[44,45]. Moreover, there is growing evidence for the importance of direct competition between strains of the same species and between species. This evidence derives from ecological modelling[46], empirical estimates of species interactions in the mouse gut[47,48] and from studies revealing the key role of bacteriocins and type VI secretion systems for ecological success in the gut[49–51]. These competitive

Box 2 | **The semantics of host–microbiota systems**

Diverse definitions abound in the study of host–microbiota systems, but the fields of ecology and evolution have a set of mostly agreed-upon definitions that can be applied consistently to avoid confusion. Here, we outline these definitions along with those of the microbiota and microbiome:

Co-evolution
Reciprocal evolutionary adaptations in different species in response to one another. If species A changes, then species B changes in response; critically, this feeds back, and then species A changes again[121].

Commensalism
Interaction between species in which individuals on one side receive net fitness benefits, whereas the other species are unaffected.

Commensal
Party in commensalism that receives benefit but has no net fitness effect on the other party.

Competition
Interaction between species in which individuals on both sides suffer net fitness costs.

Manipulation
A manipulating symbiont alters the host phenotype in such a way as to improve the fitness of the symbiont. For example, symbiont fitness may be increased by increased transmission to new hosts or increased access to resources.

Microbiome
The community of microorganisms plus the environment. In host-associated microorganisms, this translates to the microbiota plus the host environment. This follows the proposed definition[122] and logically stems from the meaning of 'biome' as a major type of ecological community. Others limit the definition of the microbiome to the genomic material of the microbiota.

Microbiota
A community of microorganisms associated with a particular environment.

Mutualism
Interaction between species in which individuals receive net fitness benefits from the interaction.

Parasitism
Interaction between species in which individuals on one side receive net fitness benefits, whereas the other species experience net fitness costs. Parasites can be members of the microbiota with ecologies similar to those of commensal and mutualistic microorganisms.

Symbiosis
Close ecological interaction between organisms (from Greek, meaning 'living with'). Examples of symbiosis include mutualism, parasitism, commensalism and more.

Symbiont
Member of a symbiosis that lives in or on the other member.

These definitions highlight that members of the mammalian microbiota are best described as symbionts rather than the commonly used commensal, because the former term is silent on the potentially varied effects on the host. Indeed, one limitation of definitions based on fitness benefits is that a single symbiont may switch, for example, from mutualist to parasite under certain conditions[123], making the classification of symbionts challenging without full knowledge of their effects[124].

conditions are predicted to lead to natural selection against symbionts — whether they are mutualists, parasites or commensals — that manipulate host behaviour (BOX 3; TABLE 1).

How do our predictions relate to current discussions of host manipulation by the microbiota? In contrast to our predictions, a recent theory paper proposed that social (specifically altruistic) behaviour in animals can be explained by the evolution of microbial manipulation[28]. However, unlike the models of parasite manipulation[39,40], this study simply assumed there was no microbial competition within hosts to disfavour a manipulating strain. It does not, therefore, challenge our predictions. If the microbial competition that occurs within the gut were accounted for in this microorganism-induced altruism model[28], then the expectation is still natural selection against manipulation[39,40]. Nevertheless, there is empirical evidence consistent with host manipulation by *Salmonella enterica* subsp. *enterica* serovar Typhimurium, mediated by the vagus nerve[52]. Bacterial infections can trigger sickness-induced behaviour in hosts[53,54]. It is challenging to demonstrate that sickness behaviour is an adaptation to combat infection rather than a by-product of compromised physiology. However, one feature — loss of appetite — may function to decrease nutrient supply to intestinal pathogens[53]. *S.* Typhimurium seems to suppress this appetite loss, which may represent manipulation of host feeding behaviour in order to counteract a potential reduction in nutrient supply[52]. Curiously, in this case, this effect seemed to improve host fitness as well as microbial fitness, though estimates of host fitness in a laboratory system may not capture fitness effects seen in the natural environment. In any case, the data appear consistent with the evolution of microbial manipulation of behaviour.

Does this *S.* Typhimurium example contradict our predictions? Consideration of *S.* Typhimurium biology suggests not; rather, this example is consistent with the predictions of when true manipulation can evolve. The evolutionary success of *S.* Typhimurium is based on its ability to transiently outcompete other species and become dominant in the gut[55,56]. This competitive dominance means that *S.* Typhimurium is not expected to be outcompeted by other species that do not invest in manipulation (TABLE 1). There remains the potential for a non-manipulating strain of *S.* Typhimurium to outcompete a manipulating one. However, this outcome would require simultaneous co-infection with multiple strains to be common, yet multiple-strain infections are rare for bacterial pathogens[57]. Importantly, the ecology of *S.* Typhimurium contrasts with the typical ecology expected in the gut microbiota. Many species exist at relatively low frequency and face competition from other strains and species over long periods, spanning many symbiont generations. These conditions are well captured by the theory[39,40], with the expectation that manipulation will often be disfavoured. Many species in the microbiota are likely to experience long-term competition, including members of the genera *Bifidobacterium* and *Lactobacillus*, which are most associated with effects on host behaviour[6–9].

Local manipulation in the host

We have discussed how the manipulation of global host phenotypes, such as behaviour, is expected only under specific conditions. However, growing evidence indicates that the microbiota affects host behaviour. What then are the alternative explanations for these effects of microbial symbionts on host behaviour? One explanation is that symbionts are naturally selected to manipulate the local gut environment, and this then influences host behaviour as a side effect. Local manipulation is predicted to be more likely than behavioural manipulation because there is a greater chance that any benefits fall preferentially on the strain that invests in manipulation (BOX 3). Potential

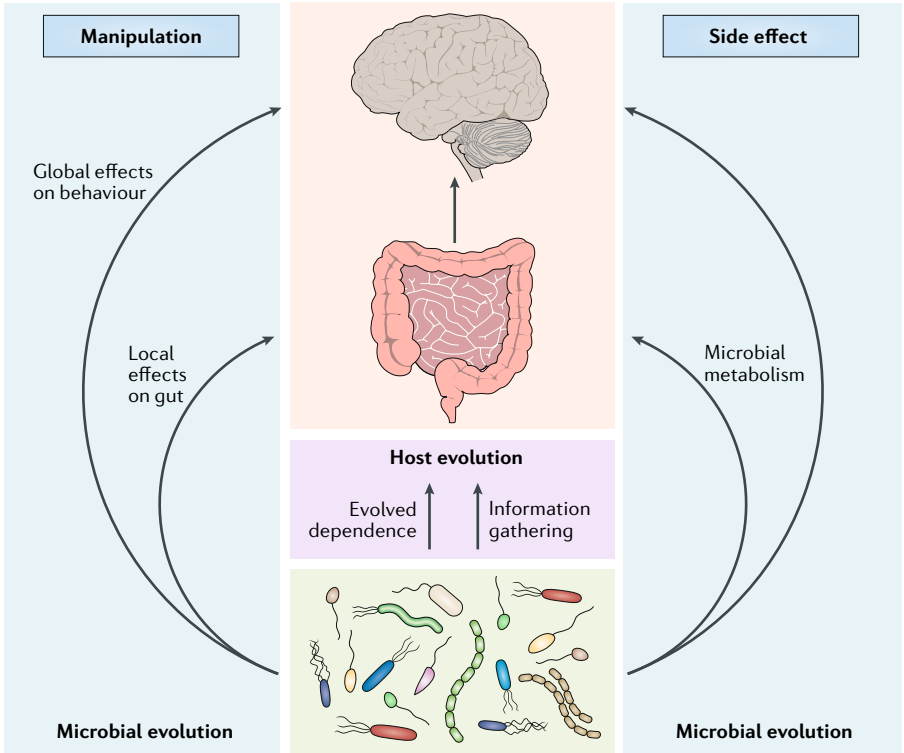


Fig. 1 | **Evolution of microbial effects on the brain.** Arrows denote the potential routes by which microorganisms may influence host behaviour. Effects driven by natural selection on members of the microbiota are shown in blue. The left-hand side captures microbial manipulation, in which case the effects on the host increase microbial fitness. Here, the microbiota–gut–brain axis arises as an evolutionary adaptation of microorganisms to influence either the gut environment (local manipulation of host physiology) or host behaviour (global manipulation of the host). The right-hand side depicts the evolution of microbial traits that affect the brain without the evolution of manipulation. For example, the evolution of the metabolism used by the microbiota to survive and divide in the gut may generate compounds, such as metabolic waste products, that affect host behaviour as a side effect. In this case, the compounds are not adapted to influence the host, and host effects are a by-product. Effects driven by natural selection on the host are shown in purple. The host may evolve to depend on the microbiota for particular functions, including nutrient provision or immune system maturation, such that a missing microbial species leads to strong physiological effects and, potentially, behavioural effects. In addition, natural selection is expected to favour hosts that use the microbiota to provide information on nutrition and health in a manner that influences feeding, foraging and sickness behaviour. In all cases, the effects of the microbiota may be due to multiple mechanisms, including the production of neuroactive chemicals that then trigger the vagus nerve or travel to the brain through the blood or lymphatic system or through effects on the immune system.

benefits of such manipulation include increased nutrient supply and decreased inhibition by attenuating host immune responses. However, to explain effects on behaviour, any manipulation must also have side effects on the central nervous system of the host. One potential route is through local changes to the enteric neurobiology of the host, which may then influence host behaviour through communication between the enteric and central nervous systems[58]. Gut bacteria can modulate intestinal motility[59] through metabolites, including short-chain fatty acids and bile acids, that affect serotonin synthesis in the host[60–63], and gut motility can in turn influence the competitive ability of certain species[64]. Moreover, a recent study found that the *Vibrio cholerae* type VI secretion system increases the strength of gut contractions in larval zebrafish and that this can displace a symbiont bacterial species[65]. This is consistent with local manipulation that provides a competitive benefit, although the example involves an acute pathogen rather than a symbiont. Whether or not such effects also have impacts on behaviour is unknown. However, effects on the enteric nervous system have the potential to modulate the mood and behaviour of the host through the gut–brain connection.

Symbiotic microorganisms may also have local effects on immune responses of the host[66,67], including reducing the inflammatory response[68,69]. However, it is not clear whether these effects represent local manipulation by the microorganisms or arise purely as a function of natural selection on the host to discriminate between different microbial phenotypes[70]. Nevertheless, the immune and nervous systems are extensively connected[71–73], not only mechanistically but also anatomically[74], such that any microbial effects on the immune system may elicit behavioural changes as a side effect (without any natural selection on symbionts to manipulate behaviour). Therefore, the possibility exists that many of the effects described in studies of the gut–brain axis may actually reflect an immune response. Consistent with this, recent findings show that colonizing mice with the faecal microbiota from patients with irritable bowel syndrome can drive anxiety-like behaviour, but only when the mice also exhibit immune activation[75].

The evolutionary basis for local manipulation by symbionts is on more solid ground than the global manipulation of host phenotypes. As such, some effects of the microbiota on host behaviour may be a side effect of local manipulation. However, even at the local scale, it is challenging to demonstrate the evolution of symbiont manipulation of the host and distinguish it from other explanations, such as the evolution of host adaptations that serve to detect and respond to particular strains and species in the gut[76].

By-products of microbial metabolism

If the microbial compounds that affect host behaviour do not arise for manipulation, then why are they produced? The simplest explanation is that they are generated as part of the metabolism that helps the microorganism to grow and divide, as occurs with metabolic waste products. Short-chain fatty acids (for example, butyrate, propionate and acetate) are key waste products made by gut bacteria that can influence gut motility[60–62], modulate host immune responses[77] and have substantial neuromodulatory effects, possibly because they function as histone deacetylase inhibitors[78]. Polysaccharide A, a component of the bacterial capsule, can also affect gut motility[79] and host immune responses[80]. In addition, microbial compounds may affect the brain. Butyrate helps maintain the integrity of the blood–brain barrier[81], which typically functions to separate the neuroactive agents of the brain and periphery[82]. Furthermore, acetate produced in the colon can cross the blood–brain barrier and directly enter the brain[83].

Compounds that function as host neurotransmitters (FIG. 2) are particularly relevant. *Lactobacillus* and *Bifidobacterium*

Table 1 | **Conditions favouring manipulation of a host by a symbiont**

Evolutionary parameter[a]	Prediction	Parasite or pathogen example[b]	Hypothetical microbiota example	Likelihood for mammalian gut symbionts
High benefit	Host behaviour affects symbiont abundance within the host and/or transmission	The fungus *Ophiocordyceps unilateralis* needs ants to move to specific elevation to develop[33] (BOX 1)	Changes in host social interactions promote microbial transmission	High
Low cost	Manipulation has limited negative effect on symbiont growth rate and survival, or manipulation is transient	Nematomorph hairworms disperse by inducing their locust or grasshopper host to jump into water; this involves only transient manipulation[137]	Microbial waste product or signalling molecule happens to strongly affect host neurophysiology. Microorganism evolves manipulation by upregulating this pathway under specific conditions	High
High within-host abundance	Abundant symbionts may benefit most if they can generate large amounts of manipulating compounds	Many manipulative parasites reach high biomass within the host, for example, *O. unilateralis* (BOX 1)	Highly abundant strain influences host behaviour. Bacteroidales strains reach high frequencies in the gut, although each strain is typically only a few percent of the total microbial cell number[50]	Low
Limited within-host evolution	Symbiont undergoes few cell divisions within the host, either owing to transient colonization or occupying a slow-growing ecological niche	*Salmonella enterica* subsp. *enterica* serovar Typhimurium, which promotes host appetite (BOX 1), only transiently infects the host[52]	Microorganism is specialist on a low-abundance nutrient in the gut	Low
Low genetic diversity	Few other genotypes — mutants, strains or species — within the niche of the symbiont, which prevents a slow-growing manipulating strain being outcompeted	*Wolbachia* strains have a diverse range of manipulative effects on insects and are intracellular, so there is little competition from other genotypes[96]	Microorganism is in a discrete compartment within the host, limiting competition	Low

[a]Evolutionary theory predicts specific conditions that favour the persistence of a manipulating symbiont[39–41]. Not all conditions are necessary for manipulation to evolve, for example, a symbiont that experiences little competition (low genetic diversity) might evolve an energetically costly manipulation trait. Critically, however, theory predicts that either limited within-host evolution or low genetic diversity is necessary for the evolution of manipulation (as they prevent a non-manipulating strain from outcompeting a slower-growing manipulating strain). [b]The best candidate examples of host manipulation come from a few types of parasite or pathogen, and we use these as illustrations. However, many parasites and pathogens do not appear to manipulate host behaviour. Indeed, some are members of the microbiota with ecologies very similar to those of commensal and mutualistic microorganisms, making them subject to the same constraints on the evolution of manipulation.

species from the human intestine are prolific producers of GABA in culture[84]. Moreover, expressing a *Bifidobacterium dentium* gene for GABA production in the mouse gut using transformed *Bifidobacterium breve* can modulate indicators of visceral pain[85]. A GABA uptake system has also been reported in *Pseudomonas fluorescens*, a plant-associated bacterium[86], and more recently, a bacterial species from the human gut microbiome, *Evtepia gabavorous*, has been shown to require GABA as a growth factor[87]. Uptake of a neurotransmitter therefore provides another potential route to influence host behaviour. In addition, bacteria seem to have an important role in activating precursors of dopamine and noradrenaline in the gut[88], and some species also synthesize serotonin, acetylcholine and histamine[89]. The production of these molecules raises the possibility that microorganism-derived neurotransmitters can bind directly to host receptors[89].

Does the production of neurotransmitters identify symbionts that have evolved to manipulate the mammalian brain? This is far from clear. First, neurotransmitters may not be produced at meaningful levels by bacteria in the gut, as studies describing bacterial neurotransmitter production are largely performed in vitro[90]. Second, it remains unknown whether lumen-produced neurotransmitters (or their precursors) can strongly influence the brain (FIG. 2). Moreover, even if microorganism-derived neurotransmitters can affect the brain, their production may well be explained by another bacterial function rather than host manipulation. Although these compounds are known as neurotransmitters in animals, they are produced not only by bacteria but also by fungi and plants[91]. Indeed, their use in multicellular species may even be explained by horizontal gene transfer from bacteria[92]. Most importantly, bacteria isolated from the environment can also produce neurotransmitters[91,93], which suggests that these compounds have a role in bacterial biology outside of the host. Determining the functions of these compounds in free-living bacteria, therefore, is an important open question, as is whether these functions translate to symbiotic species. Initial work in this area suggests functions in both core metabolism and signalling between cells[91].

The evolution of host dependence

Our focus has been on the microbiota and how microbial evolution can lead to effects on the host. However, considering how host evolution can influence the microbiota–gut–brain axis is also important. Here, there are several non-mutually exclusive routes for natural selection on hosts to affect or forge links from the microbiome to behaviour. The simplest stems from the possibility that a host behaves differently when certain microorganisms are lacking simply because the physiology of the host is compromised. Such effects can have multiple causes, but evolutionarily, they are expected from what is often called 'evolved dependence' (REFS[94,95]). When a host evolves alongside a symbiont, even a harmful one, there is the potential for it to come to

Box 3 | **Social evolution, relatedness and host manipulation**

Our prediction that the microbiota rarely manipulates mammalian hosts originates from the field of social evolution[125–128]. Social evolutionists seek to understand the origin of traits in one organism that affect the survival and reproduction of other individuals. A classic example is the sterile, and sometimes suicidal, workers of insect societies. Such phenotypes, which harm the reproduction of the individual but benefit others, are known as altruism in evolutionary biology. Altruistic traits can evolve when there is genetic similarity between the carrier and the benefiting recipients[125–127], because this means that an actor can increase the propagation of its alleles through the copies in a recipient. More specifically, the key determinant in social evolution is that of relatedness, which captures the genetic similarity between individuals at the locus that drives the altruistic trait, relative to the population average. The main way to create relatedness is family life; the evolution of sterility in workers is explained by the fact that the queen in the colony is typically the mother of the workers. This means that the workers are raising siblings and are therefore able to pass on their genetic information, even though they do not themselves reproduce.

In microorganisms such as bacteria, relatedness emerges easily by binary fission, which can create a large group of a single genotype. At the scale of such groups, cooperative phenotypes in which several bacterial cells work together are extremely common, including the production of signalling molecules, enzymes to break down complex molecules and siderophores that scavenge iron[116]. However, beyond the scale of a clonal group, competition between genotypes (through both nutrient acquisition and the many toxins used by strains to kill others) is commonly predicted and observed[115]. The challenge to host manipulation then is that multiple competing strains can benefit, whereas only the strain that actually invests in manipulation will experience the cost, putting it at a disadvantage.

The problem of competition for manipulation was realized over 15 years ago in a seminal social evolution paper that predicted a positive relationship between relatedness within a group of parasites in a host and potential investment in host manipulation[39]. Although caution is required when applying relatedness measures to microbial communities, in which many strains and species may compete and share genes[115], this prediction from the parasitology literature[39] remains relevant for the mammalian microbiota. Two sources of competition threaten to undermine a manipulating strain, one being strain diversity within its niche. If many different competing microorganisms exist, then genetic relatedness will be very low, which disfavours any trait that costs a manipulating strain but benefits all others at the scale of the host[41,76]. If a manipulating strain can prevent immigration of other strains into its niche, then the prospects for manipulation are improved. Such colonization resistance is seen in the microbiota, and some species, such as *Bacteroides fragilis*, often seem to occur as a single strain within a host[129]. However, even for such cases, a manipulating strain may be outcompeted by a second source of competitors: a mutant in the genetic background of the strain that lacks the manipulative trait. Low costs to manipulation and genetic constraints on the emergence of loss-of-function mutants may slow this process[130]. Nevertheless, the expectation is that a manipulative trait will be lost under long-term competition in the mammalian gut as any small growth cost associated with manipulation can drive the loss of a strain given the many microbial generations that commonly occur within the lifetime of a host[41].

rely on that symbiont for certain functions. For example, the wasp *Asobara tabida* has evolved to depend on the bacterial endosymbiont *Wolbachia* for normal oocyte development, even though this bacterium is commonly a parasite of insects[96].

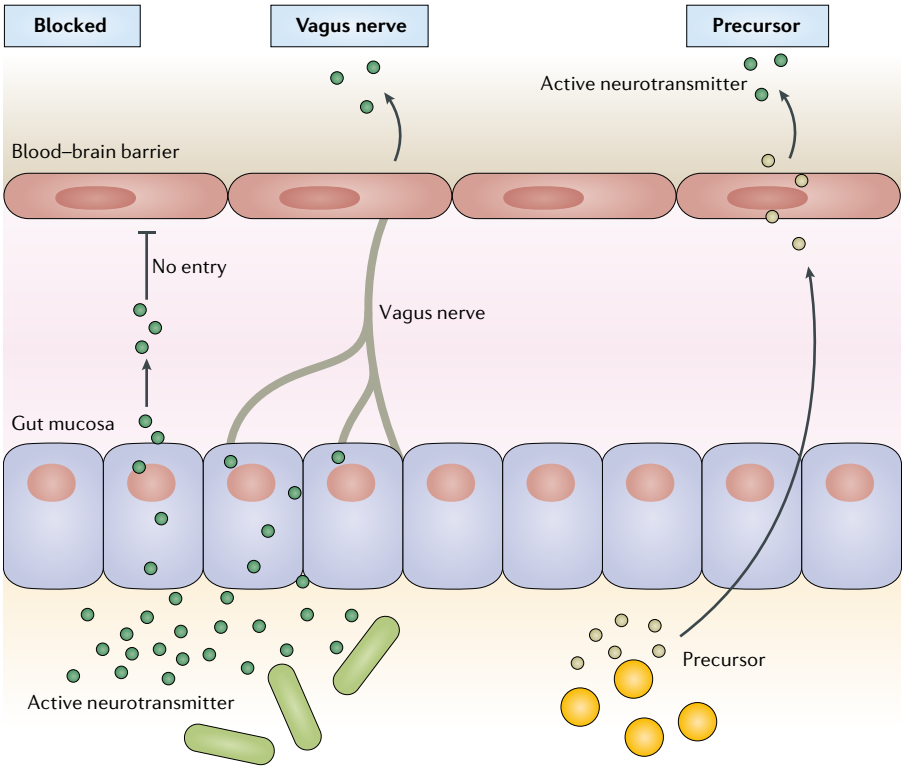
Evolved dependence may affect the nervous system, such that removing a particular microorganism creates a maladaptive physiological state that translates to behavioural effects. This can then lead to specific microorganisms having specific effects on host behaviour without any natural selection on the microorganisms to influence host physiology. Given the apparent functional redundancy of the gut microbiota[97], multiple phylogenetically diverse symbionts may complement any host dependence. Therefore, it may be the loss of the microbial trait, rather than specific microbial species, that leads to an impairment in host behaviour. More generally, evolved dependence may explain why an altered gut microbiome composition (such as in the case of germ-free or antibiotic-treated animals) is associated with behavioural changes[98–101]. If we have evolved to depend on members of the microbiota to modulate our own neurochemistry, then we might expect their absence to influence brain function.

There is also the potential for evolved dependence through the evolution of the immune system. The long evolutionary association with the symbiotic microbiota has provided many opportunities for immune regulation to evolve dependencies on bacterial phenotypes. Broadly consistent with this, the gut microbiota affects various aspects of the host immune response[77,80,102,103]. For example, microbial metabolites influence the differentiation and functioning of immune cells[104,105] and can have anti-inflammatory effects[106]. Evolved dependence has also been linked to the hygiene hypothesis, which posits a causal association between improved hygiene and the rise in autoimmune conditions[95,107]. This is based on the idea that an absence of symbiotic microorganisms or parasites leads to immune dysregulation. Most relevant here is the suggestion that the hygiene hypothesis is linked to mental health through neuroimmune connections[108]. Thus, immune processes may underpin many of the effects of the microbiome on the brain. Indeed, the bacterial genera *Lactobacillus* and *Bifidobacterium*, which are commonly associated with behavioural changes, are also known for their immunomodulatory properties[109,110]. Although the study of the microbiota–gut–brain axis has not explicitly considered evolved dependence, we believe it may prove fundamental to how the microbiome affects the brain.

Evolved dependence rests upon the idea that host physiology may come to depend upon symbionts for normal functioning. Host evolution can also generate new functions through the microbiota, and these may again affect behaviour. The gut microbiota contains much information of value to a host. When the microbiota informs on nutritional state, natural selection on the host may link the state of the microbiota to host appetite, feeding and foraging behaviour[24,111]. For example, short-chain fatty acids produced by microbial fermentation are implicated in satiety regulation[112]. Another evolved response may be sickness behaviour resulting from the spread of a pathogen within the gut (BOX 1). Furthermore, when particular symbionts provide valuable information or perform a useful function, even if just through a by-product of microbial metabolism, a host may evolve mechanisms to favour these bacterial species and thereby reinforce their effects. There are many potential routes to such host control of the microbiota by compounds secreted from the host epithelium, including both specific nutrients and antimicrobials[76]. Thus, hosts are expected to evolve to depend upon, monitor and regulate their microbiota. This evolution may readily forge and modulate links between the microbiota and host behaviour.

Outlook

There is growing evidence that the mammalian gut microbiome affects the brain and behaviour, raising the hypothesis that our microbiome has evolved to manipulate us[23–28]. However, taking stock



Examples of neurotransmitter production by bacteria

- *Bacillus* spp.: acetylcholine, dopamine and noradrenaline
- *Bifidobacterium* spp.: GABA
- *Enterococcus* spp.: histamine and serotonin
- *Escherichia* spp.: dopamine, noradrenaline and serotonin
- *Lactobacillus* spp.: acetylcholine, dopamine, GABA, histamine and serotonin
- *Lactococcus* spp.: dopamine, histamine and serotonin
- *Streptococcus* spp.: dopamine, histamine and serotonin

Fig. 2 | **How neurotransmitters in the gut lumen might influence the central nervous system.** Several neurotransmitters have been isolated from microbial species known to occur in the human gut[89] (see examples in grey box). The microbial production of neurotransmitters represents a potential mechanism to directly influence the brain and behaviour. In reality, this route is limited because most neurotransmitters, including serotonin, dopamine and GABA, cannot typically breach the protective blood–brain barrier[82,131]. Alternative modes of action include the possibility that microorganism-derived neurotransmitters affect the brain through the vagus nerve and its afferent neurons[132]. Another option is that precursors of neurotransmitters cross the blood–brain barrier[133,134] and are then converted into active neurotransmitters. For example, gut bacteria can influence the metabolism and availability of the serotonin precursor tryptophan[135]. This may affect serotonergic signalling in the central nervous system as tryptophan concentration in the blood plasma has been shown to correlate with brain serotonin levels[136].

of both data and evolutionary theory casts serious doubt on this hypothesis. The theory predicts that manipulation is most likely when the manipulative trait has low cost and high benefit for the manipulating bacteria and, critically, when there is limited competition from non-manipulating strains (TABLE 1). This last condition does not seem easily satisfied in the diverse microbial ecosystem of the gut. We should not then assume that our microorganisms are our puppeteers. Instead, the behavioural effects of the microbiota might be better explained as a side effect of either local manipulation of the host environment or the microbial metabolism needed to grow and survive in the gut. Moreover, it is clear that hosts can evolve to depend upon the microbiota and use it to respond to nutritional and disease states, thereby cementing a link from symbionts to host physiology.

Our perspective has implications for both understanding and manipulating how the microbiota affects behaviour. We predict that microbial compounds that influence host physiology, such as neurotransmitters, typically evolve either because of their local impacts on host physiology (local manipulation) or as a by-product of natural selection on microorganisms to grow and compete within the microbiota (FIG. 1). Local effects on mucus production[41,113], the inflammatory response[56] and gut motility[64,65] all have the potential to influence microbial strains differently in ways that are important for evolutionary success. However, the clearest evidence for local manipulation currently comes from acute pathogens, such as *Salmonella* strains and *V. cholerae*, rather than from beneficial or commensal symbionts. The demonstration of local manipulation by symbionts requires more than simply showing effects of a microorganism on host physiology. Critically, local manipulation is also predicted to increase the competitive ability of the manipulating strain in the gut, in contrast to behavioural manipulation, which is not expected to improve local competitive ability (TABLE 1). For symbionts that spend a long time in the host, this predicts that a locally manipulating strain will outcompete an otherwise isogenic strain lacking the manipulative trait. Moreover, this competitive benefit must arise from effects on host physiology.

Arguably, the simplest explanation for microbial traits that influence host behaviour is that they are a by-product of the way that microorganisms grow and compete in the gut (FIG. 1). Similar to local manipulation, this scenario predicts that the production of a host-affecting compound will provide a competitive advantage to the strain that carries it. However, in contrast to local manipulation, the advantage will occur independently of the effects on host physiology. This implies that any advantage can also be observed experimentally ex vivo (to the extent that the experiment can capture the growth conditions in the host). What kinds of molecule help a microorganism to compete but might also affect host physiology? Many candidates exist. We have focused our discussion on metabolic products such as short-chain fatty acids, which are known to strongly influence the physiology of host cells[60–62,77]. However, bacteria produce vast numbers of compounds whose effects on gut physiology are currently unknown[114].

Metabolic waste products are just one source of compounds released by bacteria. To compete in any community, microorganisms produce a wide variety of compounds that influence the survival and division of other cells (BOX 3). These include enzymes that break down complex molecules, biosurfactants, siderophores that scavenge iron, diverse toxins that inhibit other microorganisms, extracellular polymeric substances (including carbohydrates and DNA), molecules that function as electron acceptors and molecules that function in cell–cell communication (for example, quorum sensing)[115,116].

Such compounds are potential candidates for influencing host physiology because they can be released in large quantities and, moreover, have often evolved because of their physiological effects on other cells. For example, iron is a key currency for both host and microbial cells[117], and bacterial siderophores that scavenge iron are known to affect epithelial cell physiology[118].

A focus on microbial ecology also has implications for the goal of engineering the microbiota[76]. It has been suggested that probiotic strains can be used to improve mental health outcomes[19]. A major challenge with many probiotics is getting a strain to establish itself in a new community[76,119]. However, a focus on naturally occurring human symbionts should circumvent this challenge. If we are correct that host-affecting traits are accompanied by a competitive advantage, then probiotic strategies should be viable in the sense that the strains can compete and establish themselves in communities. Another limitation of probiotic strategies is the tendency to seek a single strain to provide a given benefit. In reality, the benefits that the microbiota provides to the host, such as protection against pathogens, can arise from the interactions of multiple species within a community[120]. If this is also true for behavioural effects, then we will need to embrace the full ecological complexity of the microbiota in order to understand the gut–brain axis.

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Competing interests
The authors declare no competing interests.