PHILOSOPHICAL TRANSACTIONS B

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Review

Cite this article: Eisthen HL, Theis KR. 2016 Animal–microbe interactions and the evolution of nervous systems. *Phil. Trans. R. Soc. B* **371**: 20150052.
http://dx.doi.org/10.1098/rstb.2015.0052

Accepted: 30 August 2015

One contribution of 16 to a discussion meeting issue 'Homology and convergence in nervous system evolution'.

Subject Areas:
behaviour, evolution, microbiology, neuroscience, physiology

Keywords:
holobiont, microbiome, microbiota, symbiosis

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Animal–microbe interactions and the evolution of nervous systems

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Animals ubiquitously interact with environmental and symbiotic microbes, and the effects of these interactions on animal physiology are currently the subject of intense interest. Nevertheless, the influence of microbes on nervous system evolution has been largely ignored. We illustrate here how taking microbes into account might enrich our ideas about the evolution of nervous systems. For example, microbes are involved in animals' communicative, defensive, predatory and dispersal behaviours, and have likely influenced the evolution of chemo- and photosensory systems. In addition, we speculate that the need to regulate interactions with microbes at the epithelial surface may have contributed to the evolutionary internalization of the nervous system.

1. Introduction

We live in a microbial world. Since their origin, multicellular organisms have been co-evolving with microbes, a collection of organisms including bacteria, archaea, fungi, protozoa and viruses. Microbes colonize the gut and external surface of animals, as well as some reproductive organs. Some animals even have additional, specialized organs that harbour selected groups of microbes. In general, despite the ubiquity of microbes, associations between animals and microbes are not random [1]. Animals do not merely tolerate microbes; we possess complex suites of adaptations to provide beneficial microbes with food, suitable habitats and protection from other microbes.

The contributions of microbes to animal biology—including human biology—are significant [2,3]. Many readers will be aware that current estimates suggest that our bodies contain 10-fold more microbial cells than human cells, and up to 500-fold more microbial genes than human genes [4]. More concretely, the cumulative mass of the human microbiota is 1–2 kg [4], which is sobering considering that the average human brain has a mass of approximately 1.5 kg [5].

Given the relative proportion of microbes and microbial genes in animal bodies as well as the fidelity of these associations across animal generations, researchers have begun to refer to such ensembles as 'holobionts' and to suggest that holobionts are valid units of selection in animal evolution (e.g. [6,7]). This idea has come to be known as the hologenome model of evolution [7–9]. This change in focus has the potential to upend the way we think about evolutionary change, often defined as a change in gene frequency over time. Brucker & Bordenstein [10], for example, argue that we should be thinking of animal evolution as a change over time in the frequencies of genes in the nucleus, in organelles and in microbial symbionts.

With the recent proliferation of research on animals and their associated microbes, biologists are increasingly learning that microbes play key roles in physiological processes of animals, including in neural function. In this paper, we briefly review three examples of current relationships between microbes and the neurobiology and behaviour of animals: chemosensory detection of microbial products (§2), the role of microbes in foraging and predation

(§3), and microbial influences on cognition and social behaviour (§4). In §5, we propose that microbes may have played a key role in the evolution of nervous systems.

2. Chemosensory detection of microbial products

Microbes and microbial products play important roles in animal behaviour. At arguably the simplest level, animals can taste and smell chemical compounds produced by microbes and use this sensory information to avoid pathogenic microbes, as indicators of the presence of food sources or conspecifics, and even as chemical signals.

The nematode *Caenorhabditis elegans* eats soil bacteria, but it must be discriminating: it has to avoid pathogenic microbes while selecting those that are palatable and beneficial. One pathogen avoided by *C. elegans* is *Serratia marcescens*, which, if ingested, produces compounds that kill the nematode and dissolve its eggshells. Nematodes detect *Serratia* using the paired AWB chemosensory receptor neurons [11]. Of the 302 neurons in *C. elegans*, 16 paired neurons function as chemosensory receptors; the AWB cells, along with two other pairs of cells, detect cues that mediate avoidance responses [12]. Although the ASJ cells are normally involved in attraction responses and dauer formation [12], they can also mediate avoidance responses induced by bacteria. Specifically, when at high density in low-oxygen environments, *Pseudomonas aeruginosa* is pathogenic to *C. elegans*. Under these conditions, the bacterial metabolites phenazine-1-carboxamide and pyochelin are detected by the ASJ neurons, which then produce neuromodulators that alter activity in adjacent neurons. This activity leads the animal to seek higher oxygen environments, away from potential pathogens [13]. Thus, it appears that *C. elegans* possesses distinct sensory-neural pathways for generating appropriate behavioural responses to specific cues from potential microbial pathogens.

The fruit fly *Drosophila melanogaster* is strongly repelled by geosmin, a volatile odorant produced by *Penicillium* fungi and *Streptomyces* bacteria. These microbes grow on decaying fruit and are lethal to *Drosophila* [14]. Thus, fruit flies must discriminate fruit that is at the optimal level of ripeness: when fruit is overripe by most human standards, the yeast that grow on it provide food for *Drosophila* and are highly attractive [15], but fruit that is so overripe that it smells of geosmin must be avoided. Indeed, the odour of geosmin abolishes the normal attraction to vinegar and other food-related odorants; it also inhibits oviposition [14]. Stensmyr *et al*. [14] have shown that geosmin is detected by a specific class of antennal olfactory receptor neurons, ab4B, that appears to be narrowly tuned to detect only this molecule. These neurons project exclusively to the DA2 glomeruli in the antennal lobe, the first olfactory processing region in insect central nervous systems. The projection neurons from the DA2 glomeruli are also narrowly tuned to geosmin, suggesting the presence of a labelled line for detecting geosmin. Finally, the authors found geosmin-specific olfactory receptor neurons in seven other species of drosophilids, strongly suggesting that microbial products have shaped the evolution of the *Drosophila* nervous system.

In addition to warning of the presence of dangerous microbes on substrates, microbial products can provide information about the infection status of conspecifics, including potential mates, in a wide diversity of organisms [16–18]. Mice avoid conspecifics that are ill [19], and recent evidence suggests that mice use receptors that normally function as part of the innate immune system to smell compounds characteristic of bacterial infection in conspecifics. Mammalian leucocytes possess specialized receptors for formylated peptides, which are released by bacteria as metabolic by-products. Formylated peptides attract leucocytes to sites of infection (reviewed in [20]). Interestingly, two formyl peptide receptors are expressed in the vomeronasal organ, an accessory olfactory organ, in mice [21,22]. *In vitro*, the receptors can be activated by formylated peptides and other infection-related molecules [22]. More recent work shows that the vomeronasal organ is essential for mice to distinguish the odours of infected and uninfected conspecifics [23]. Taken together, these results strongly suggest that formyl peptide receptors in the vomeronasal organ warn mice of the presence of infected conspecifics.

Microbial products are not always aversive to animals, as anyone who likes the taste of miso or a ripe cheese, or the smell of wine or baking bread can attest. Stable flies (*Stomoxys calcitrans*) select oviposition sites based on the presence of particular bacteria, likely detected from a distance by odorant cues [24]. In addition, settlement cues for marine invertebrate larvae, such as barnacles and polychaetes, are often produced by biofilms of microbes either on stable sea floor substrates or on adult conspecifics [25–28]. Given the critical importance of choosing an appropriate habitat—immobile benthic animals that settle far from conspecifics will not reproduce—cues produced by microbes play a critical role in the life history of these animals.

Although the details of the mechanisms whereby marine invertebrate larvae detect settlement cues are still being worked out, studies with drosophilids have recently revealed remarkably precise and robust systems for detecting and responding to volatile odorants produced by microbes. In general, drosophilids are strongly attracted to volatiles produced by yeasts, their main food source—so much so that the Solomon lily (*Arum palaestinum*) is pollinated by drosophilids attracted to its scent, which contains volatiles normally produced by yeasts rather than by plants [29]. In addition, *D. melanogaster* is attracted to hydroxycinnamic acids, antioxidant molecules that can prolong life when included in the diet. Although they cannot directly detect the presence of these compounds, flies are acutely sensitive to the presence of the volatile odorants 4-ethylphenol and 4-ethylguaiacol, which are produced by a variety of yeasts as a result of metabolizing hydroxycinnamic acids. The dedicated olfactory receptors used to detect the compounds differ between adults and larvae, but in both cases the animals are attracted to and feed on substrates containing these odorants; adults also preferentially lay eggs on such substrates [30].

Odorants emitted by symbiotic bacteria also play important roles in what used to be called intraspecific communication among animals, although given the importance of microbes in producing some of these odorants, 'intraspecific' is not quite the appropriate term. Nevertheless, we here use the word 'cues' to refer to sensory stimuli that provide information about the animals that emit them, sometimes to the detriment of these animals. 'Signals' also provide information about the animals that emit them, but signals have been selected over evolutionary time to efficaciously communicate this information between senders and receivers, both of whom benefit

from the exchange. To the extent that their contributions to variation in hosts' scents accurately reflect underlying traits, symbiotic microbes can broadcast information about their hosts to other animals through chemical cues and signals [31,32]. Animals rely heavily on chemical signals to communicate their species, group and individual identities, as well as information about their sex and reproductive state [33,34], and odour-producing symbiotic microbes have been broadly hypothesized to contribute to animal chemical signalling [31,32,35–37].

Among insects, symbiotic microbes can produce volatile compounds that are used as host-specific pheromones (reviewed in [36,37]), although some microbes can also convert pheromones into repellents (e.g. [38]). The best-understood example involves the aggregation pheromone of the desert locust (*Schistocerca gregaria*). Adult desert locusts can be solitary or gregarious, and during the transition to gregariousness, locusts undergo dramatic genetic, hormonal and behavioural changes that render them capable of forming massive swarms that can decimate crops over large distances [39]. After adopting the gregarious morph, locusts aggregate in response to volatiles that are present in the faeces of adult males, but not females or younger animals; the key component of the aggregation pheromone is guaiacol [40,41]. Morphologically distinct sensillae on the antenna respond to sex pheromones, plant odorants and organic acids, and the aggregation pheromone [42]. Notably, the aggregation pheromone inhibits responses in sensillae to plant odorants [42]. Further, the percentage of antennal lobe neurons that respond to guaiacol increases with age and is higher in gregarious than solitary adults [43]. Guaiacol is not produced by locust cells but by gut bacteria, likely as a product of metabolizing vanillic acid derived from lignans in the locusts' diet [44–46]. Surprisingly, the microbe is not unique, as several different microbes, including *Pantoea agglomerans*, *Klebsiella pneumoniae* and *Enterobacter cloacae*, can produce guaiacol within locusts [45]. This example of apparent coevolution between an animal and its symbiotic microbes raises many interesting questions. For example, if guaiacol is produced by gut bacteria, why is it produced by adult males but not females? Do sex hormones play a role? In addition, it would be interesting to determine whether the presence of guaiacol-producing gut bacteria changes with age, and if so whether the bacteria contribute to age-related changes in olfactory sensitivity to guaiacol. Desert locusts have the potential to serve as a powerful model system for understanding the behavioural and physiological processes that contribute to selection of gut microbes as well as their influence on host nervous systems.

Among mammals, preliminary evidence indicates that symbiotic microbes substantially contribute to their hosts' complex signature scents [31,32,37]. For example, many mammals communicate using specialized scent glands, which are known to harbour diverse communities of odour-producing microbes [31]. In original cultivation-based studies of the Indian mongoose (*Herpestes auropunctatus*), bacteria from scent glands were shown to produce a suite of short-chain fatty acids (SCFAs) typical of scent secretions. In addition, a broad-spectrum antibiotic eliminates both the bacterial communities and scents from these glands, and mongooses respond to both scent secretions and experimental mixtures of SCFAs in a manner suggesting that they communicate information about signallers' individual identities to receivers [47,48]. More recent cultivation-independent studies of spotted hyaenas (*Crocuta crocuta*) and striped hyaenas (*Hyaena hyaena*) showed that the bacterial and SCFA profiles of the two hyaena species differ and that the two profiles covary within each hyaena species. Further, among spotted hyaenas, the two profiles are social group-specific, and reflect sex and reproductive state among members of the same social group [49–51]. Similar patterns are evident among mammals that communicate via urine marking as well. For example, microbes associated with the urine marks of male African elephants, *Loxodonta africana*, are responsible for the production of urinary ketones and alcohols believed to signal male musth status [52,53]. Similarly, microbes in the urine marks of house mice (*Mus musculus*) appear to function in communicating individual- and genotype-specific information among competitors and prospective mates [54,55].

3. Role of microbes in neural mechanisms of foraging and antipredator behaviour

At an arguably more complex level, microbes play important roles in foraging and antipredator behaviour in a broad spectrum of animals. For example, one of the best known anti-predator defensive molecules is the neurotoxin tetrodotoxin (TTX). TTX blocks the pore of voltage-gated sodium channels, preventing neurotransmission in almost all multicellular animals. TTX is sufficiently toxic that sympatric reef fishes gain protection from predation by visually mimicking pufferfish [56], and in pufferfish and many other animals, TTX is produced by symbiotic bacteria [57]. Although possessing TTX confers obvious advantages to pufferfish, it also requires physiological adaptations beyond providing a suitable habitat for TTX-producing bacteria: all eight genes coding for voltage-gated sodium channels possess mutations that confer TTX resistance [58], and toxic pufferfish possess a novel gene duplication that created a protein involved in the transport and accumulation of TTX [59]. TTX serves not only in defence, as it is used by both blue-ringed octopus (*Hapalochlaena maculosa*) and planocerid flatworms to envenomate prey [60,61].

Beyond toxicity, symbiotic microbes can function in antipredator defence by providing camouflage. The Hawai'ian bobtail squid (*Euprymna scolopes*) possesses a ventral light organ that provides counterillumination, preventing the squid from casting a shadow that could attract the attention of predators lurking below. The light organ is colonized by luminescent *Vibrio fischeri*, and juvenile squid possess complex adaptations for selecting *V. fischeri* from among the many species of environmental bacteria that can enter the light organ [62,63]. Interestingly, eye-specific genes are expressed during development of the light organ, and the organ expresses phototransduction molecules and produces electrophysiological responses to light [64,65]. The function of light detection in the organ is not yet understood, but it may allow the animal to exclude mutants that fail to luminesce [66] or may enable the squid to match the ambient illumination level. In either case, the relationship with symbiotic *Vibrio* has clearly led to evolutionary adaptations in the squid nervous system.

In some cases, microbes hijack normal antipredator behaviour, manipulating the nervous systems of animals to

produce outcomes that are favourable to the microbe but not the host. For example, the fungal pathogen *Ophiocordyceps unilateralis* infects the nervous system of ants (*Camponotus leonardi*), leading the ants to attach themselves to leaves for several days until a fruiting body erupts from the ant's head, scattering fungal spores [67]. Other *Ophiocordyceps* species infect other species of *Camponotus*, and the ability to manipulate the ants' behaviour is unique to its naturally occurring pathogen [68,69]. Another well-known example involves infection of rats by the protozoan *Toxoplasma gondii*, which causes rats to become attracted to cat urine, greatly increasing their risk of predation by the parasites' definitive host [70]. Describing more examples of parasite manipulation of host behaviour is beyond the scope of this review, and the interested reader is directed to recent special issues of *The Journal of Experimental Biology*, 216(1), Jan. 2013, and *Integrative and Comparative Biology*, 54(2), July 2014 for more information.

Like bobtail squid, cardinalfish (*Siphamia versicolor*) possess a ventral light organ containing luminescent bacteria (*Photobacterium mandapamensis*). Instead of using their light organ for antipredator defences, however, cardinalfish use their light organs to attract planktonic prey. Emission of light is under neural control, as the organ can be occluded using a retractable shutter [71]. Like the bobtail squid, cardinalfish can also determine the amount of luminescence in the light organ: the organ abuts the lower jaw, which is largely translucent, and the ventromedial portion of the eyes protrudes into the buccal cavity. Thus it appears that the fish can directly see the luminescence of the light organ [71]. Interestingly, olfactory preference tests suggest that cardinalfish are attracted to chemical cues emitted by *P. mandapamensis* [72], which colonize the light organ from the external environment. Although symbiotic bacteria are necessary for proper development of the light organ in bobtail squid [73], the bacteria that colonize the light organ of cardinalfish are not necessary for its development [74]. Taken together, these studies indicate that the association with luminescent bacteria has powerfully shaped the nervous system and anatomy of cardinalfish.

In addition to facilitating feeding behaviour, symbiotic microbes may also contribute by influencing dietary decisions. In a recent essay, Alcock *et al*. [75] postulate that gut microbes may manipulate host feeding behaviour by generating cravings for particular foods that they themselves can readily access as energy sources, or by inducing dysphoria in their hosts until those particular foods are consumed. Gut microbes could accomplish this by affecting host taste receptor expression, vagus nerve activity or neuroendocrine profiles, for example by producing dopamine, serotonin or peptides that influence satiety [75]. Evidence for these hypotheses is to date circumstantial, but elucidating and evaluating the influences of gut microbes on host feeding behaviours should be a research priority moving forward.

4. Influence of microbes on cognition and social behaviour

Recent studies have revealed that microbes contribute to surprisingly complex behaviours, including cognition and social behaviour. Almost all such studies to date have involved laboratory mice and rats, and compare germ-free animals (raised in an environment with no microbes) to both specific pathogen-free and conventionally raised animals.

Interestingly, germ-free mice show reduced exploration of unfamiliar conspecifics and spend less time near conspecifics than do control animals (e.g. [76,77]). In addition, rearing mice in germ-free environments results in an exaggerated physiological response to stress, which can be reversed by restoration of normal microbial communities or even just the bacterium *Bifidobacterium infantis* [78]. Paradoxically, several studies indicate that germ-free mice show decreased anxiety and increased exploratory behaviour and suggest the existence of a sensitive period after which introducing microbes does not reverse the effects of germ-free rearing (e.g. [79,80]). Gareau *et al*. [81] have suggested that this discrepancy might be reconciled if the increased exploratory behaviour in germ-free mice is due to deficits in working memory rather than decreased anxiety, leading animals to greater levels of exploration; further, studies using varying measures of anxiety obtain differing results (e.g. [76]).

The neural correlates of altered behaviours in germ-free mice are rarely clear, but their nervous systems are altered in ways that are consistent with changes in anxiety, learning and social behaviour. For example, germ-free mice show widespread changes in gene expression in the amygdala [82]. Such mice also show reduced expression of brain-derived neurotrophic factor in the dentate gyrus and CA1 layer of the hippocampus [79,81], as well as in the amygdala and cingulate cortex [82,83]. Expression of *N*-methyl-D-aspartate receptor subunits NR1 and NR2A is also reduced in the hippocampus [78], and of NR2B in the central amygdala [79], in germ-free mice. Finally, such mice also show increased turnover of dopamine, noradrenaline and serotonin in the striatum [83]. While such studies are in their infancy, it is clear that the microbiota plays a powerful role in normal neural function as well as in behavioural and neural development in mammals (for recent reviews, see [5,84–86]). Nevertheless, research to date has largely focused on a small number of mammalian species, and broader comparative studies are needed.

5. Could microbes have played a role in internalization of nervous systems?

Given our long history of co-evolving with microbes, as well as the examples described in §§2–4, it seems clear that microbes have played a role in nervous system evolution in at least some specific cases. In addition, we propose that microbes played a role in a major event in nervous system evolution: the internalization of nervous systems in animals derived from ancestors that possessed nerve nets.

The nature of the first neurons is not clear, nor do we agree how many times neurons may have originated [87,88]. Data from demosponges suggest that proto-neurons were sensory cells embedded in the external epithelium [89–91]. These proto-neurons may have signalled to each other or to neighbouring epithelial cells in paracrine fashion, and adaptations for improved signalling fidelity, including neurites and synapses, might have evolved later [92]. The signalling molecules used by these proto-neurons were likely peptides [93]. The molecular components required for the synthesis and release of neuropeptides are present in demosponges, as are homologues of eumetazoan neuropeptide

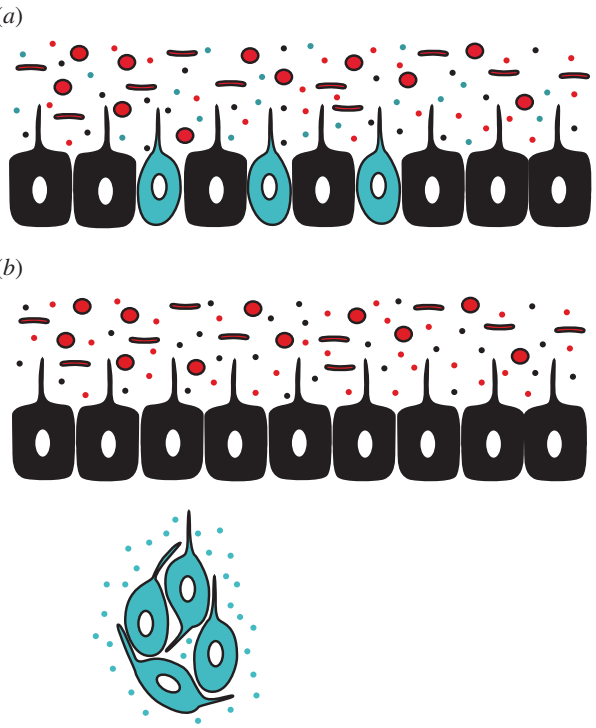

Figure 1. Illustration of a hypothesis concerning the role of microbes in nervous system internalization. Proto-neurons are depicted in blue, epidermal cells in black, and both coccus- and rod-shaped microbes are depicted in red. The smaller blue, black and red dots represent peptides and other small molecules produced by the three classes of cells. (*a*) The ancestral condition, in which proto-neurons are distributed among epidermal cells. In this state, metabolic products and signalling molecules released by microbes and anti-microbial peptides released by epithelial cells may interfere with interneuronal signalling. (*b*) In animals with an internal nervous system, neuronal signalling is largely protected from interference; in addition, epithelial cells can regulate the composition of the microbial community and microbes can signal to epithelial cells without interference from neuronal molecules. (Online version in colour.)

receptors [91]. Thus, even before nerve nets arose, proto-neurons, peptide signalling molecules and receptors for these signalling molecules were likely present.

Among animals with nerve nets, peptide neurotransmission may predominate ([94], but see [95]). Many neurobiologists think of neuropeptides as functioning as modulators that activate relatively slow processes; nevertheless, fast, peptide-gated ion channels that are related to acid-sensing ion channels (ASICs) are present in a variety of animals, including cnidarians [96] and molluscs [97,98]. In addition, although neurotransmission in ctenophores is poorly understood, large numbers of fast, peptide-gated ion channels are coded in the genome of *Pleurobrachia bachei* [99], and the genome of the demosponge *Amphimedon queenslandica* contains at least one putative ASIC (NCBI reference sequence: XP_011405396.1). Thus, the earliest neural signalling molecules in animals with nerve nets may have been peptides that elicited relative rapid responses from neighbouring neurons.

Although it is not yet clear how many times internal nervous systems arose from animals with nerve nets [93], we propose that microbes may have played an important role in driving the evolution of internalized nervous systems. We can envision three ways in which microbes may have contributed to internalization of the nervous system; these hypotheses are not mutually exclusive.

First, as illustrated in figure 1, nervous systems may have become internalized to optimize signal fidelity. In general, neuropeptides bind to G protein-coupled receptors (GPCRs), evolutionarily ancient receptors that predate the split among plants, fungi and eukaryotes [100,101]. Two of the five major families of GPCRs—the rhodopsin-like and glutamate-like GPCRs—are present in animals without nervous systems, such as *Amphimedon* and *Trichoplax*, as well as in ctenophores and cnidarians [102], and could have functioned as the earliest receptors for neural signalling molecules. A basic property of GPCRs is that a single receptor can couple to different intracellular signalling pathways such that different ligands can influence different physiological processes. In other words, GPCRs are inherently promiscuous [103], although in some cases GPCRs have co-evolved with particular ligands to a high degree of specificity (e.g. [104]). Thus, if GPCRs served as receptors for early neuropeptide signalling molecules, these receptors were vulnerable to interference by other molecules.

Molecules that could create such interference are abundant at the body surface. Epithelial cells produce peptides to regulate microbial communities in and on animals' bodies. Indeed, *Hydra* use antimicrobial peptides to control species-specific microbiomes [105,106], suggesting that anti-microbial peptides date to at least the last common ancestor of cnidarians and bilaterians and may be widespread across the animal kingdom. Given that the neurons that comprise nerve nets are interspersed among these epithelial cells, the peptides produced by epithelial cells have the potential to interfere with neuronal GPCRs. Further, microbes also produce peptides as metabolic products as well as for use in intraspecific signalling and interspecific competition (for recent reviews, see [107–109]), providing yet another source of potential interference with neural signalling. Finally, many bacterial products are detected by GPCRs (e.g. [110]). As a result, pathogenic bacteria have evolved mechanisms to modulate or block activity of animal GPCRs to avoid detection [111], a process that again could compromise neural signalling. Taken together, these observations suggest that nervous systems may have been internalized to protect neuropeptide receptors from 'noise' due to the presence of antimicrobial peptides and microbial products.

Second, a core group of 16 families of peptidases are ubiquitous and as old as life [112], and neuropeptides that are released into the mucus at the epithelial surface are vulnerable to degradation by these enzymes. This creates obvious problems for paracrine signalling, as peptides that are broken down before they reach their target are poor signals. Further, degradation poses an additional problem: when degraded by peptidases, neuropeptides can break down into smaller products that can also activate or antagonize either the parent receptor or receptors for other peptides [113]. Perhaps nervous systems were internalized to avoid problems related to degradation of neuropeptides, which can lead to ineffective signalling and receptor noise.

A brief example may serve to illustrate the kind of cross-talk that we envision among receptors, peptides and peptidases. The nervous systems of vertebrates contain many peptides that possess a C-terminal-RFamide, similar to the invertebrate neuropeptide FMRFamide; such peptides are often referred to as FMRFamide-like peptides (FLPs). FLPs interact with a variety of receptor subtypes [114], and the same receptor can interact with many different FLPs

(e.g. [115,116]). For example, frog nervous systems contain members of peptide families that are widely distributed among vertebrates and could be considered FLPs, including gonadotropin inhibiting hormone [117–119]; kisspeptin [120]; met-enkephalin and other opioid peptides [121,122]; prolactin-releasing peptide [123]; and neuropeptide Y, peptide YY and pancreatic polypeptide [124]. The skin of frogs also contains many anti-microbial peptides [125], including several FLPs [126]. Finally, frog skin also contains numerous peptidases [127]. Thus, it seems that unless the nervous system were internal to the epidermis, the multiple FLPs in both neurons and skin cells, multiple FLP receptors on neurons, and multiple peptidases produced by skin cells could all interfere with both neural signalling and epithelial–microbial signalling in frogs.

Third, nervous systems may have become internalized to protect them from invasion by microorganisms. Neural receptors on the surface of an animal are risky, as viruses can gain entry to neurons through receptors; to cite some familiar examples, rabies, herpes and measles viruses all enter neurons through cell-surface receptors [128–130]. Once inside a neuron, viruses can use synapses to rapidly spread from cell to cell, potentially invading large portions of the body [131,132]. Perhaps nervous systems were internalized to protect the organism from infection. Notably, the enteric nervous system of the gastrointestinal tract, which must be in constant communication with the luminal environment [133,134], is protected from gut microbes by a variety of defences, including intestinal epithelial tight junctions, a resistant mucosal barrier and broad and targeted immune responses [134,135].

6. Conclusion

At a time when the influence of microbes is starting to be recognized as fundamental to all aspects of animal biology, it is critical that we consider microbial impacts on the function and evolution of animal nervous systems. All animals necessarily interact with environmental and symbiotic microbes, as well as metabolic products of microbes, and the outcomes of these interactions can be beneficial or detrimental to animals. In this paper, we have described how these interactions have shaped animals' chemosensory and photosensory systems in ways that promote the avoidance of pathogens and predators, the location of important resources including food and suitable mates, and the effective signalling of key information among animal conspecifics. We also introduced a burgeoning line of inquiry regarding the effects of symbiotic microbes on mammalian neurodevelopment, cognition, anxiety and social behaviour, and call for comparative studies on these subjects. Finally, we discussed how beneficial phenotypes conferred by symbiotic microbes to their animal hosts can require concomitant adaptations in animals' nervous systems to accommodate and manage these residents and suggested that a key event in neural evolution—the internalization of the nervous system—may have resulted from selection pressures involving microbes.

Authors' contributions. H.L.E. and K.R.T. developed the ideas presented here, wrote the article and approved the final version to be published.

Competing interests. We have no competing interests.

Funding. This work was funded by the US National Science Foundation (IOS-1354089 to H.L.E. and subawards from DBI-0939454 to H.L.E. and K.R.T.).

Acknowledgements. Thanks to Nicholas Strausfeld and Frank Hirth for organizing the meetings from which this paper emerged. In addition, we thank Emma Coddington, Stephen Thomas and Patric Vaelli for useful discussions of the ideas presented here.

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