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How bacteria impact host nervous system and behaviors: lessons from flies and worms

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Keywords:

Host-pathogen interactions; behavioral immunity; *Drosophila melanogaster*; *Caenorhabditis elegans*; neurons; bacteria.

Abstract:

Behavior is the neuronally controlled, voluntary or involuntary response of an organism to its environment. An increasing body of evidence indicates that microbes, which live closely associated with animals or in their immediate surroundings, significantly influence animals' behavior. The extreme complexity of the nervous system of animals combined with the extraordinary microbial diversity are two major obstacles to understand, at the molecular level, how microbes modulate animal behavior. In this review, we discuss recent advances in dissecting the impact that bacteria have on the nervous system of two genetically tractable invertebrate models, *Drosophila melanogaster* and *Caenorhabditis elegans.*

Main text

Microbes influence animal's behavior

Microorganisms which appeared on our planet more than 3.5 billion years ago, later co-evolved with animals. From this cohabitation, a significant interdependency arose between hosts and their surrounding or associated microbes, which had profound effects on metazoan biology, fitness, reproduction, and physiology. It is therefore no surprise that **allochthonous** and **autochthonous** microorganisms have also important influences on animal behavior [1, 2]. While a large number of microbes are pathogenic and pose a threat to an animal's survival, others, such as those forming the symbiotic **microbiota**, play beneficial roles for the host [3] [4]. Hence, when navigating in their environment, animals benefit from being able to differentiate between beneficial and harmful microorganisms. They can, for example, taste and smell chemical compounds produced by microbes and use this sensory information to avoid pathogenic microbes. Timely detection of harmful bacteria is expected to be beneficial in many ways for the host. By decreasing exposure to a pathogen, it increases its survival chance and limits the spreading of the threat to its sibling and progeny. It also reduces energetic expenses by preventing the activation of the costly immune response (**Box 1**). In other circumstances, microorganisms are precious sources of information indicative of favorable sites for foraging, laying offspring, as well as nursing and raising them [5, 6]. Microorganisms can also alter the behavior of the host once they have infected them [7]. While some of these behavioral changes are seen as side effects inherent to the modulation of host homeostasis, genetic studies have demonstrated that others result from a direct molecular dialog between the microorganism and the host nervous system. They could represent a non-canonical immune response aimed at reducing the consequences of the infection for the host or its offspring. Dissecting these peculiar inter-organism interactions is certainly an important field of research for the coming years. However, the enormous diversity of microbes that cohabit with animals and the highly complex organization of the eukaryotic nervous system complicate the task. Elucidating the causal relationship between host-microbe interactions and behavioral changes can undoubtedly benefit from the use of relatively

simple and genetically tractable models. In the last years, studies in two invertebrate model systems, *Drosophila melanogaster (D. melanogaster)* and *Caenorhabditis elegans (C. elegans)*, have not only unraveled the extreme pleiotropic modes of interactions that take place between microorganisms and the nervous system of animals but also begun to reveal the nature of the microbial elicitors, the type of neurons that detect them, and the behavioral consequences associated with their reciprocal interactions.

In this review, we will provide an overview of recent achievements in both animal models, with a specific emphasis on the interactions between bacteria and the host nervous system. The first part will be devoted to the mechanisms involved in bacteria detection by neurons. We will then discuss current knowledge related to the modulation of the immune response by neuronal inputs. Lastly, we will illustrate how some "immune" proteins are also implicated in neuronally-controlled host behaviors under infected or physiological conditions.

***C. elegans* detects and avoids pathogenic bacteria through its sensory neurons**

The nematode *C. elegans* is present in soils where it encounters a variety of bacteria species [8]. As expected for a bacterivorous animal, it thrives in media containing innocuous food sources *such as Escherichia coli (E. coli)*, but escapes from those contaminated with pathogenic species which can be life-threatening [9]. For an animal constantly foraging in bacteria and using a bacterial lysate as food source, the classical self/non-self paradigm does not strictly apply (**Box 1).** How then is the worm distinguishing between innocuous and pathogenic bacteria? In laboratory conditions, *C. elegans* avoids *Serratia marcescens* (*S.m.*) that can digest the worm's eggshell and produce deadly compounds. This lawn-avoidance behavior is mediated by two head olfactory neurons exposed to the environment (AWB), that are part of the amphid, the largest chemosensory organ of the nematode (**Box 2**). Triggered by the *Serratia*-produced serrawetin W2, this lawn-behavior requires the Toll-like receptor TOL-1 [10]. In this prey-predator race, *C. elegans* also escapes from nematicide molecules produced by *Streptomyces*. Avoidance of *Streptomyces*-produced dodecanoic acid, requires the expression of the GPCR, SRB-6 in a subset of head

(ASH, ADL, ADF) and tail (PHA, PHB) **chemosensory neurons** [11]. Unexpectedly, in two-choice assays between innocuous bacteria and *S. m. or Pseudomonas aeruginosa (P.a.), C. elegans* is initially attracted to pathogenic bacteria. Only after some hours of exposure, trained animals learn to avoid pathogens and exit the bacterial lawn. This delay may correspond to a period during which nematodes learn to avoid odors of pathogenic bacteria and generate memory of the encounter. Other data, discussed below, suggest that it results from the development of the infection and it is associated with host damages. Exposure to pathogens also upregulates the expression of serotonin in the ADF chemosensory neurons. Serotonin functions through MOD-1, a serotonin-gated chloride channel expressed in sensory **interneurons**, to promote olfactive aversive learning [12]. Recent studies dissected the molecular and cellular bases and characterized the neuronal network underlying this behavior [13-15]. In sum, in parallel to food-seeking behaviors that allow them to search and identify beneficial bacteria, nematodes perceive and react to biotic stress via dedicated neuronal circuits.

C. elegans senses local gas concentration to detect bacteria

Modifications that microorganisms cause to their environment are also a source of information for the worm. Local concentrations of oxygen are important cues used by *C. elegans* to move in its environment, a phenomenon called **aerotaxis** behavior [16]. Usually attracted by low O_2 and high CO_2 concentrations that are indicative of bacteria-enriched substrates, worms use gas-level sensing to mount protective avoidance behavior. Under high bacterial density conditions, *P.a.* produces secondary metabolites such as phenazine-1-carboxamide and pyochelin. Detection of these metabolites by ASJ neurons (amphids) activates the production of the TGF-β family member DAF-7 that, in turn, inhibits DAF-3 signaling in the adjacent RIM/RIC **interneurons** [17]. This neuronal activation leads the worm to seek higher oxygen environments, away from potential pathogens. Bacterially produced CO_2 is another cue used by nematodes to escape pathogens. Defect in CO_2 detection by gas-sensing BAG neurons positively correlates with a defect in avoidance of *Serratia* [18]. In this context, the TOL-1 receptor and downstream signaling events are

required to specify the fate of BAG chemosensory neurons. In addition, *C. elegans*, which unlike most metazoans lacks Nitric Oxide synthase and consequently cannot synthesize NO, uses this gas as an environmental cue to avoid *P.a.* [19]. This response is mediated by the ASJ **chemosensory neurons** and requires NO-mediated activation of receptor guanylate cyclase and cyclic nucleotide-gated ion channels (DAF-11 and GCY-27). *P.a.* mutants deficient for NO production fail to elicit avoidance. These results demonstrate that gases produced by microbial respiration are important molecular cues used by nematodes to avoid metabolically active pathogens. However, since both pathogenic and harmless microbes respire aerobically and produce CO_2, the sole presence of this gas does not indicate whether the microbes that produced it are harmful. *C. elegans* might reserve this option to feed on attenuated or dead microbes that would otherwise be pathogenic and probably integrate other cues to distinguish pathogenic from non-pathogenic bacteria (Figure 1).

Bacterial detection mainly requires the olfactory and gustatory systems of D. melanogaster

D. melanogaster lives primarily on rotten fruits populated by microbes that synergistically ferment organic substrates to produce active compounds and metabolites [20] [21]. Detecting these chemosensory molecules helps the flies to find nutrient-rich food, to select hospitable zones for egg-laying, and to avoid ecological niches contaminated with pathogens. In *D. melanogaster*, tastants and volatiles are detected by hundreds of gustatory and olfactory neurons distributed on multiple body parts including the antennae, maxillary palps, proboscis, wing margins, legs, and ovipositor [22](**Box 3**). Some constitutive elements of the bacterial cell wall and membrane can be directly sensed by these neurons. Detection of bacterial **lipopolysaccharide** by the esophageal bitter neurons via the **TrpA1** receptor triggers feeding and oviposition avoidance [23]. When applied onto wing margins or legs, bacteria cell wall **peptidoglycan (PGN)** induces grooming behavior [24]. Unpublished data from our group indicate that PGN can also be detected by fly gustatory bitter neurons via the classical immune **pattern recognition receptor** of the peptidoglycan recognition proteins (**PGRPs**) family [25] (A.M., L.K., J.R. unpublished data).

As for *C. elegans*, the fly olfactory system plays a key role in adapting behavior to the presence of bacteria. *D. melanogaster* larvae fed with the opportunistic pathogen *Erwinia carotovora caratovora (E.c.c.)* drastically reduce food intake [26]. This feeding blockage requires the universal olfactory co-receptor *Orco* and **TrpA1**. Geosmin, a volatile odorant produced by some fungi and bacteria acts as a strong fly repellent that can override innate attraction to vinegar and other food-related odorants [27]. Its activity is mediated by a single class of neurons expressing the **odorant receptor** 56a (Or56a) and which target the DA2 glomerulus in the antennal lobe. Carnivore feces are enriched in bacteria that produce phenols. Phenol detection by Or46a olfactory neurons present in the fly palp triggers oviposition aversion [28]. Activation of the geosmin and phenol circuitry is sufficient to induce a reduction in oviposition suggesting that they are powerful signals for the presence of potential infectious sites containing harmful microbes. Consistently, these signals have been shown to also be aversive in other insect species. Besides protecting flies from detrimental bacteria, the olfactory system can also mediate fly attraction to microbes. Indeed, the detection of bacterial short-chain fatty acid by Or30a neurons acts as an orexigenic signal for the larvae [29]. Optimal identification of a given bacteria species presumably requires the integration of multiple sensory modalities. Consistently, when given the choice between a sugar only and an *E.c.c.*-contaminated solution, flies are first attracted by the bacteria and after few hours repulsed by it. While the initial attractive phase depends on the olfactory Gr63a neurons, the second repulsive phase requires the bitter taste Gr66a neurons. Interestingly, by providing a food source for the flies, *E.c.c.* facilitates the potentiation of bitter neurons allowing the avoidance behavior to be established [30]. Altogether, these data demonstrate the roles played by the fly and worm **sensory neurons** in detecting environmental bacteria and mounting behaviors to either avoid them if they are toxic or, on the contrary, to move towards them and feed on them when they are beneficial (Figure 2).

Intestinal bacteria impact C. elegans behavior

The gut **microbiota** is mainly composed of bacteria species that are either neutral or beneficial for the host. However, the ingestion of pathogenic bacteria together with environmental and genetic variations can lead to **dysbiosis** with detrimental consequences for the host. How these quantitative or qualitative changes in gut bacteria populations alter host behavior is a growing area of research. For the nematode that feeds on bacteria and empties its intestine content within minutes, the existence of gut **microbiota** is still debated (**Box 1**). However, some reports have shown that some gut bacteria can affect *C. elegans* behavior. To avoid being killed by *P.a.*, worms move away after a few hours of contact with the pathogen. Although this delayed response has been attributed to olfaction-dependent aversive learning (see above), it has been proposed that lawn avoidance is a consequence of the damages caused by the ingested bacteria. This is supported by the lack of lawn avoidance of non-pathogenic bacteria or avirulent mutants of *P. aeruginosa*. Furthermore, the avoidance behavior observed in *C. elegans* fed with *E. coli* producing dsRNA that inactivates genes required for fundamental cellular activities, also reinforces the hypothesis of cellular damage sensing [31]. As in the case of pathogen-avoidance, noxious RNAi-dependent avoidance also engages a serotonergic circuit, since it is reduced in the serotonin biosynthetic mutant *tph-1*. Another model has emerged from reports studying bacteria sensing by the gut epithelium [32, 33]. Mutant worms defective in either pharyngeal pumping (*phm-2*) or defecation motor program (DMP) present an increased gut bacterial load that is correlated with an avoidance response. Since inhibition of gut colonization abrogates the escape response, bacterial colonization and, consequent bloating of the intestine could be perceived as a danger signal by the worm. Increased avoidance caused by the *phm-2* mutation also requires TPH-1-mediated serotonin biosynthesis but is independent of NPR-1-mediated neuropeptide signals [32]. Moreover, the avoidance caused by increased colonization in the *DMP* mutants requires NPR-1 and the two neuropeptides FLP-18 and FLP-21, although serotonin biosynthesis plays a negligible role here [33]. It remains unclear what might cause this discrepancy.

Moreover, in contrast to the aforementioned results [17], the rapid chemosensation of *P.a.* derived phenazine-1-carboxamide and pyochelin, which leads to the induction of DAF-

7/TGF-β in ASJ neurons, does not correlate with the avoidance behavior [34]. Instead, bloating of the intestinal lumen induces the avoidance behavior via modulation of both DAF-7/TGF-β and the GPCR NPR-1 neuroendocrine pathways, which results in a preference for O_2 and thus in pathogen avoidance behavior [34]. Since there is no general agreement on how gut-associated bacteria trigger avoidance in *C. elegans*, further work will be needed to determine the relative contribution of gut bacteria sensing and/or gut bacteria host damage induction to this phenomenon. The consideration of the timing seems to be crucial to understand this behavior which certainly results from the integration of several inputs. Whereas bacteria-induced innate processes are expected to be rapid, slower kinetics would be expected for a behavior secondary to changes in the internal state of the infected animal.

Gut bacteria-dependent neuropeptides affect *C. elegans* behavior

In the complex network of influences that *P.a.* can exert on *C. elegans* behavior, insulin-like neuropeptides also play a role. When exposed to *P.a.*, worms present an upregulation of the neuroendocrine peptide INS-11 in the intestine. By inhibiting the expression of *ins-6* in ASI neurons and serotonin synthesis in ADF neurons, INS-11 negatively regulates aversive learning behavior [35, 36]. The decrease in learning abilities upon pathogen exposure might appear as a disadvantage for the host. However, aversive learning behavior has to be balanced with the need to resume eating and produce progeny. If the balance is to strongly tilted to one side or another, nematodes might be unable to recognize and avoid pathogens, or they might starve and become less fertile. Consistently, *ins-11* loss-of-function mutants that are inefficient in seeking new sources of food, consume more energy and have fewer offspring (Figure 1). Host behavior can also be affected by neuropeptides produced by gut bacteria. Released by the commensal *Providencia*, the bioamine tyramine is converted to octopamine by the *C. elegans* tyramine β-hydroxylase. Octopamine, in turn, targets the OCTR-1 octopamine receptor on ASH nociceptive neurons to modulate an aversive olfactory response. Food choice assays demonstrate that worms are preferentially colonized by *Providencia* and that this selection bias requires bacterially

produced tyramine and host octopamine signaling. Hence, a neurotransmitter produced by a gut bacterium can mimic the functions of the cognate host molecule and override host control of a sensory decision, and thereby promotes fitness of both the host and the microorganism [37].

Bacteria-derived compounds can be sensed internally by** D. melanogaster **neurons

Contrary to *C. elegans*, the existence of the gut **microbiota** in *D. melanogaster* is well established and studies involving **gnotobiotic** and **axenic** flies are possible [38, 39]. While the pleiotropic roles played by gut-associated bacteria in fly development and physiology are amply documented, their influence on behavior only begins to be elucidated [40]. By acting via the olfactory system, gut-associated bacteria can influence fly preferences in food-seeking and choice of egg-laying sites [41-43]. However, internal bacteria can also alter neuronally controlled behaviors independently of the sensory system. When compared to their conventionally reared sibling, **axenic** flies show enhanced locomotion [44]. Gut recolonization by *Lactobacillus brevis* is sufficient to bring locomotion back to normal levels. Genetic and biochemical data demonstrated that bacteria-produced xylose isomerase is critical to sustaining normal fly locomotion. Although the exact mechanisms involved remain unclear, xylose isomerase mediates its effects by inactivating the CNS neurons that produce **octopamine**. The same neuromodulator is central to another bacteria-induced behavior modification in the fruit fly. When mated females are infected by bacteria, they reduce their oviposition to spare the energy required to fight infection or to prevent progeny development in a non-favorable environment [45]. Previous work has revealed that during an immune response, the detection of bacteria-derived **PGN** by **PGRP** receptors triggers an **NF-κB**-dependent production of **antimicrobial peptides** (**AMP**) in immune cells [46] (**Box 1**). Surprisingly, the same bacterial elicitor and the same signaling pathway regulate the reduction of female oviposition following bacterial infection [45]. **PGN** sensing and **NF-κB** activation in few octopaminergic neurons in the fly brain are sufficient to modulate egg-laying in infected females [47]. Therefore, a unique bacteria cell wall constituent and a common host signaling cascade are used in immune cells to mount

an immune response and in brain neurons to control fly behavior following infection. While it is well established that gut-borne PGN can cross the gut epithelium to reach circulating hemolymph, its mode of access to the brain remains unknown [48, 49]. Interestingly, the biogenic amine **octopamine** was also shown to mediate the effects that the endosymbiotic *Wolbachia* bacteria can exert on *D. melanogaster* male aggressivity [50]. Finally, pathogens can also modulate host behavior to their advantage. By changing the pheromone levels in the **frass** of the flies they infect, *Pseudomonas entomophila* attracts healthy flies leading to their contamination and favoring pathogen dispersal [51]. Fly mating behavior can also be influenced by bacteria that are associated with the host. Isogenic *D. melanogaster* populations prefer mating with partners with similar **microbiota**. Although it has been proposed that gut-associated bacteria influence mating preferences by changing host sex pheromone levels, the exact mechanism is still unclear [52] (Figure 2).

C. elegans neurotransmitters modulate the immune response

Historically seen as a role for immune cells only, mounting a specific and efficient response against pathogens clearly requires the contribution of non-immune tissues. In this context, the nervous system appears essential to tune immunity according to physiological contexts and to coordinate behavioral and immune responses upon microbial exposure. Work in *C. elegans* has revealed how neurotransmitters modulate the immune response [53, 54]. For instance, serotonin synthesized in cephalic ADF **chemosensory neurons** signals to rectal cells. The signaling in these posteriorly located cells, which depend on the Gα-protein GAO, suppresses the immune response and limits pathogen clearance rate [55]. Dopamine produced in CEP neurons acts through the ASG neurons to inhibit intestine immune signaling upon *P.a.* exposure [56].

Endogenously produced by RIC neurons, **octopamine** binds to OCTR-1, an **octopamine** receptor, to suppress immunity [57]. Indeed, OCTR-1 signaling in ASH and ASI **sensory**

neurons down-regulates the translation of immune genes and the unfolded protein response (UPR) pathway in non-neuronal tissues [58-60]. However, this specific aspect linking immune regulation and non-canonical UPR is still debated and has been shown to depend on nematode culture conditions [61, 62]. Since **octopamine**-producing neurons are inhibited when exposed to *P.a.* but not to the harmless *E. coli*, this neuronal break could allow the worm to adapt its immune response to the nature of encountered bacteria. More generally, these negative regulations could function to mitigate immune response or to restore protein homeostasis after infection. This is well illustrated for the GPCR-encoding *npr-8* gene which is expressed in amphid neurons (AWB, ASJ, AWC) and negatively regulates the expression of collagen genes in the worm cuticle [63]. Thus, NPR-8 production influences host defense against pathogens by modulating the physical barrier. However, in contrast to these previous examples, the neuro-immune connection can also reinforce host defense. In *C. elegans* infected by *Staphylococcus aureus*, neuronally produced acetylcholine functions in an endocrine fashion to engage muscarinic receptors in the intestinal epithelium and induce Wnt-dependent expression of host defense genes [64]. The establishment of an adapted antibacterial enteric response depends also on neuro-immune interactions that took place early in life, during developmental processes. Expression of *orln-1* in the olfactory AWC neurons is critical for olfactory receptor differentiation during larval development. Loss-of-function mutant analysis indicates that ORLN-1 acts non-cell autonomously to repress p38 MAPK-dependent immune responses in the intestine [65]. These data suggest that low activity of neuronal ORLN-1 de-represses the p38 MAPK PMK1 pathway to prime the immune response in the intestine, thus allowing to handle challenges by bacterial pathogens encountered during larval development (Figure 1). Thus, the worm nervous system not only detects pathogenic bacteria leading to avoidance behavior, but also modulate the activation of canonical immune pathways in non-neuronal cells in both physiological and infected conditions.

Neuronal signaling influences fly cellular immunity

In contrast to *C. elegans*, *D. melanogaster* possesses circulating immune cells that can engulf and eliminate invasive bacteria (**Box 1**). These professional phagocytes called plasmatocytes are mainly produced by the hematopoietic organ called **lymph gland** and released into the blood**.** Their numbers and properties vary in response to developmental and environmental cues some of which are of neuronal origin [66]. Activin-β, a TGF-β family ligand that is expressed by **sensory neurons** of the peripheral nervous system, regulates the proliferation and adhesion of hemocytes. Agonist-mediated activation and transient silencing of these **sensory neurons** affect resident hemocyte numbers and localization [67]. Environmentally-derived neuronal signals also control fly hematopoiesis. Activation of fly olfactory neurons leads to the secretion of GABA from neurosecretory cells into the circulation. Upon binding to its metabotropic receptors expressed on hematopoietic progenitors, GABA regulates the balance between maintenance and differentiation of these progenitors in the **lymph gland** [68]. One candidate upstream sensor is the odorant receptor Or42 although the ligand(s) involved is still unknown. Neurons have also been implicated in connecting environmental gas level cues to myeloid differentiation. Both the inactivation of CO_2-sensing neurons and the stimulation of hypoxia-sensing neurons lead to an increase of Hypoxia-inducible factor-α in downstream neurons. In turn, these neurons release the JAK/STAT ligand Unpaired-3 which triggers Insulin-like peptide-6 production by the fat body cells. Once released into the circulation this hormone promotes crystal cell (one blood cell type) differentiation in the **lymph gland** [69]. It would be of significant interest to decipher if and how bacterial infection directly modulates the activation of these olfactory and gas-sensitive neurons that function upstream of hematopoietic differentiation.

New roles for old friends: The multiple roles of NF-κB and antimicrobial peptides in neuronal tissues

The interplay between the immune and the neuronal systems is also revealed by the growing number of proteins historically considered as immune effectors or regulators for which a function in the nervous system has been observed. An example of how immune

protein activity has extended beyond host defense has been described for the pro-inflammatory cytokine IL-17. When worms are exposed to 21% O_2, they tend to aggregate. Impairment of IL-17 receptors in RMG **interneurons** induces defects in O_2-dependent social behaviors. IL-17 can act directly on neurons to modulate their responsiveness to presynaptic input and circuit sensitivity to O_2. Knowing that O2 level-dependent aggregation and bordering of *C. elegans* are influenced by the presence of bacteria, IL-17 signaling may have played a role in ancestral nervous systems in the regulation of behavioral responses to bacteria [70, 71]. Similarly, a role in the regulation of neuronal function and behavior by immune proteins has been reported in *the fruit fly*. In *D. melanogaster* neuromuscular junctions, perturbation of neurotransmitter receptors in the muscle cell enhances neurotransmitter release from the motor neuron, a phenomenon called presynaptic homeostatic potentiation (PHP). The immune **pattern recognition receptor PGRP-LC** and some downstream pathway components of the **NF-κB**/IMD pathway are required presynaptically to regulate PHP. However, the **NF-κB**/IMD signaling bifurcates downstream of the **PGRP-LC** receptor to achieve immediate modulation of the presynaptic release apparatus via the TGF-β activated kinase (Tak1), and prolonged maintenance of the homeostatic response via the transcription factor **NF-κB**/Relish [72, 73]. Since PHP has no obvious links with bacterial immunity, it is possible that PGRP-LC is activated at the synapse by an endogenous ligand. Besides the regulation of neuronal function, the **NF-κB**/Relish protein has also been involved in sleep regulation. Together with other immune effectors, it turns out to be upregulated upon sleep deprivation [74]. Consistently, flies mutant for **NF-κB**/Relish exhibit a reduced sleep period and, unlike their wild-type siblings, are unable to increase sleep upon bacterial infection [75]. Since both phenotypes are rescued by providing **NF-κB**/Relish in fat body cells, it is likely that **NF-κB**-regulated genes produced by fat body cells modulate sleep behavior. As mentioned above, the canonical **NF-κB** antibacterial pathway functions in octopaminergic neurons to regulate oviposition. Although **antimicrobial peptides** (**AMPs**) seem dispensable for this response (A.M., L. K. J.R. personal communication), they have been implicated in other neuronal activities. Nemuri, a peptide with antimicrobial properties expressed in few brain neurons is induced

upon sleep deprivation. Flies in which Nemuri is overexpressed in neurons survive infection by *S. marcescens* or *S. pneumoniae* better than control flies. Nemuri could therefore act by prolonging sleep to promote fly survival after infection [76]. Moreover, gain-of-function experiments suggest that when expressed in neurons (Drosocin) or glial cells (Metchnikowin) some **AMPs** could contribute to resilience to sleep deprivation [77]. Finally, genetic inactivation of *Achilles*, a neuronal gene showing a highly rhythmic expression pattern, results in dramatically high levels of immune response effectors, including **AMPs** [78]. As a result, flies are more resistant to immune challenge with bacteria. Other biological effects of immune genes on nervous function include memory formation. Diptericin B and the bacteria sensor GNBP-like3 are upregulated following behavioral training. Knock-down experiments revealed that while they both regulate long-term memory, Diptericin B functions in the head fat body and GNBP-like3 in neurons to prevent memory deficit [79]. **AMPs** are produced as a result of immune stimulation, so it can be imagined that the formation of memories related to the event that determined their production may be beneficial for the fly. In contrast to previous examples, recent reports revealed that **AMPs** may also play a role in neurodegenerative diseases. Indeed, **AMP** accumulation has been shown to induce neuronal damage in flies. Hyperactivation of innate immunity in the brain as a result of genetic mutations or bacterial injection causes neurodegeneration linked to the neurotoxic effects of **AMPs** [80]. With age, flies present an **NF-κB**-dependent constitutive **AMP** gene expression in glial cells which is accompanied by progressive neurodegeneration and locomotion decline [81]. Similarly, aging-associated expression of the **AMP** NLP-29 causes dendrite degeneration in *C. elegans*. By activating the orphan GPRC NPR-12, NLP-29 induces autophagy to mediate aging-associated dendrite degeneration, a mechanism also observed after infection by the fungus *Drechmeria coniospora* [82]. This finding supports the existence of signaling pathways possibly linking microbial defense to degeneration. The growing number of immune proteins and pathways involved in neuronal functions raises the broader question of how precisely should one delineate the range of phenomena to be considered strictly as immune

response, and whether the definition of an immune cell should be expanded or reconsidered.

Concluding remarks and future perspectives

In the thousands-of-pieces puzzle of the network that underlies microbe interactions with the nervous system of animals, work in recent years, focusing mostly on a few specific bacteria species and animal models, has begun to assemble some of the pieces. While some trends are emerging, such as the role of **octopamine** in mediating many of these interactions, our knowledge remains fairly rudimentary, with many unanswered questions (**see Outstanding Questions**). There is a good chance that, as the number of bacteria species studied increases, the number of mechanisms and molecules involved increases in concert. And this without mentioning other non-bacterial parasites, such as viruses or fungi, some of which are also capable of altering the behavior of the hosts they infect [7]. Much work, therefore, still lies ahead**.** It can be hoped that some of the insights gained using studies in *C. elegans, D. melanogaster,* and other invertebrate models will be useful for elucidating how bacteria impact on cognitive functions and fundamental behavior patterns in higher eukaryotes.

Figure legends:

Figure 1: Interactions of bacteria with the *C. elegans* nervous system. Some bacteria species produce metabolites that upon sensing by the amphid sensory neurons trigger pathogen avoidance. Depending on the species, the bacterial trigger and the host sensing neurons have not always been identified. Different mechanisms underlie pathogen avoidance; these include avoidance learning behavior and aerotaxis-related avoidance behavior. Avoidance can also be triggered after the ingestion of the pathogen. How these gut-associated bacteria induce worm escape is not yet clear. While some authors propose that intestine bloating is the trigger, others state that tissue damages occurring from intestinal infection are a key component of aversive learning response. Integration of

Commenté [MF1]: The figure files were labeled reversely – Fig 1 was drosophila and Fig 2 c-elegans. Please let me know whether it's ok to relabel the figure files (so that Fig 1 is c-elegans and fig 2 drosophila)

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several inputs including the aforementioned ones might allow the animal to fine-tune its reaction towards bacteria.

Figure 2: Interactions of bacteria with the *D. melanogaster* nervous system. Environmental bacteria produce metabolites and volatiles that can be directly sensed by the fly olfactory and gustatory neurons. The same is true for constituents of the bacteria cell wall such as lipopolysaccharide (LPS) and peptidoglycan (PGN). Subsequent activation of sensory neurons induces host behavior changes such as bacteria avoidance or modulation in food intake, egg-laying rate or grooming. Some bacteria cell wall components such as PGN can enter the body cavity and directly act on brain octopaminergic neurons to modulate the egg-laying rate.

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Glossary:

Aerotaxis: oxygen-dependent migration.

Allochthonous bacteria: non-resident bacteria species that live in the animal environment and can eventually infect it.

Antimicrobial peptides (AMPs): small molecular weight proteins with broad spectrum antimicrobial activity against bacteria, viruses, and fungi. These evolutionarily conserved peptides are usually positively charged and have both a hydrophobic and hydrophilic side that enables them to be both soluble in aqueous environments yet able to penetrate lipid-rich membranes.

Autochthonous bacteria: resident bacteria species that live in association with the host. Typically, some species that form the microbiota.

Axenic: germ-free.

Dysbiosis: imbalance in host-associated microbial communities that can be associated with diseases.

Frass: excrement or other refuse left by insects and their larvae.

Gnotobiotic: germ-free animals that have been associated with controlled bacteria species.

Interneurons: nerve cells that relay impulses between projection neurons, for instance between sensory neurons and motor neurons.

Lipopolysaccharide (LPS): present in almost all Gram-negative bacteria, LPS is the major outer surface membrane component. It consists of a polysaccharide region that is anchored in the outer bacterial membrane by lipid A. Its detection by *ad hoc* PRR triggers an immune response.

Lymph gland: larval organ in which most of the *D. melanogaster* hemopoietic cells are generated.

Microbiota: communities of microorganisms that live in or on an animal. The species that live in the intestine form the gut microbiota.

Odorant receptor (OR): insect odorant receptors are transmembrane ionotropic receptors that may also use metabotropic signaling Most insect ORs function in the presence of another shared receptor known as Orco.

Octopamine: monoamine closely related to mammalian norepinephrine. This neurotransmitter which acts through G-protein coupled receptors regulates many behaviors in invertebrates.

NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells, is a protein complex that controls the transcription of DNA. NF-κB is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, bacterial or viral antigens. Three NF-κB members exist in flies (Relish, Dorsal, and DIF). It has not been found in *C. elegans*

Pattern-Recognition-Receptors (PRRs): germline-encoded host sensors, which detect molecules typical for the microbes. They are proteins expressed mainly in cells of the innate immune system but also some neuronal cells.

Peptidoglycan (PGN): polymers of sugars and amino acids that form a mesh-like layer outside the plasma membrane of most bacteria, thus constituting the cell wall.

Peptidoglycan-Recognition-Receptors (PGRPs): receptors that play essential roles in triggering the antibacterial innate immune response in invertebrates. Although their main ligand is the bacteria-derived peptidoglycan some family members can be activated by other bacterial ligands. They are also present in mammalian proteomes [25].

Sensory neurons: nerve cells responsible for converting external stimuli from the environment into internal electrical impulses.

TrpA1: transient receptor potential cation channel, subfamily A, member 1, is an ion channel located on the plasma membrane of many human and animal cells. It is one of the most promiscuous TRP ion channels with many identified ligands such as LPS.

Box 1: *D. melanogaster* and *C. elegans* anti-bacterial responses

***D. melanogaster*.** To study anti-bacterial responses in *D.* melanogaster, flies are typically either infected by wounding the abdomen cuticle with a contaminated needle or by feeding on bacteria-contaminated medium. Genetic and genomic studies revealed the pivotal role for the TOLL and IMD signaling cascades in *D. melanogaster* antimicrobial response [79, 80]. These signaling pathways can be activated locally in exposed epithelia as well as systemically in the fat body. Activation of these pathways depends on the detection of bacteria-derived peptidoglycan by PGRP sensor proteins [22]. These pathways culminate in the translocation of NF-κB to the nucleus leading to infection-specific upregulation of AMPs dedicated to clear the infection. The cell-mediated immune system relies on blood cells and is induced by epithelial damage and detection of foreign particles in the hemocoel [81]. Hemocytes seal epithelial wounds, encapsulate and terminate parasites and engulf apoptotic corpses or bacteria. *D. melanogaster* has three major lineages of hemocytes: plasmatocytes with phagocytic capacity, crystal cells that are implicated in the melanization process and lamellocytes that encapsulate large foreign bodies.

***C. elegans*:** Bacteria can infect and kill nematodes [82]. *C. elegans* feeds on bacteria, and their standard food in laboratory settings is a slow replicating strain of *Escherichia coli*. To expose nematodes to other bacteria such as *Pseudomonas aeruginosa* or *Serratia marcescens*, the animals are deposited on a plate seeded with the desired microbe; accordingly, in some respects, these infections can be considered as natural. This protocol allows worms to seek for the bacteria, to flee the bacterial lawn or to make choices between two strains or species. During the feeding process, bacteria are pumped in the pharynx then grinded and a bacterial lysate fills the intestinal lumen. Infections are principally characterized by bacteria able to survive and proliferate within the intestinal lumen, leading to the precocious death of the animal. Septic injury is not a model extensively used. The main pathways involved in the antibacterial responses in *C. elegans* are the TGF-β, the p38/MAPK and Wnt pathways and the responding cells are those exposed to the threat. TOL-1, homologue of the *D. melanogaster* Toll has not been found to

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Commenté [MF3]: Is this the standard food in the lab, or also in ethological situations? (if the latter, is it always the typical food? If not, a qualifier such as "often" would be in place)

Commenté [MOU4R3]: Yes it is

C. elegans immune response. When analyzing the upstream events leading to defense activation, it appeared that detection of modified self rather than interactions with microbe-associated molecular patterns like LPS or PGN is used by this invertebrate. Indeed, chemicals perturbing central processes like translation are inducers of the immune response. The same is true with the ToxA bacterial toxin that impairs the ribosomal activity [83].

Box 2: *C. elegans* nervous system

The nematode does not possess a so-called brain even though nearly a third of its somatic cells are neurons, with adult hermaphrodite *C. elegans* possessing 302 neurons (https://www.wormatlas.org/). Thanks to its invariable developmental pattern and intensive studies including reconstruction based on serial EM micrographs, the exact position of each neuron as well as the neuronal connectivity are known. Each neuron has its own name using a system of three to four letters (e.g. AFD). Most of the neurons are located in the head around the pharynx in an area called the nerve ring, others are longitudinal, around the vulva or close to the tail. There are currently 32 designated chemosensory neurons and their functions can range from proprioceptors to oxygen sensors or chemosensors. Four chemosensory organs have been described with amphids (in the heads) and phasmids (in the tail) being the largest chemosensory organs of worms. These specialized group of cells are made of support and socket cells, which define a sensillum that is an opening through which dendrite of sensory neurons are exposed to the external milieu. Interestingly, the chemosensory neurons directly contact neuronal circuits (interneurons and motoneurons) dedicated to forward or backward movement. However, chemosensory neurons are exclusive and one cell controls either attraction or repulsion, but not both. Chemosensory neurons can be dedicated to volatile compounds (1-octanol, diacetyl) as well as water-diluted molecules (NaCl) with more than one specific receptor expressed in the dendrite of each neuron. The candidate proteins to mediate chemosensation are GPCRs and the nematode genome encodes around 1.300 of them. Contrary to chemosensation, the neurons necessary for the response to oxygen are exposed to the nematode internal fluids and the receptors are guanylyl cyclases combined

with hemes. Pheromone sensing is also present with a complex chemical lexical. Thus, nematodes navigate in the environment, integrate cues from oxygen and carbon dioxide levels, are attracted toward putative food source, can sense the population density, are repulsed when exposed to noxious chemicals and are capable of learning with a memory lasting several days.

Box 3: *D. melanogaster* olfactory and gustatory systems

Gustatory system

D. melanogaster can detect basic taste including sugar, bitter, salt, and acid. The fly taste system is distributed over the whole body. Dose-dependent activation of different taste cells provides a simple mechanism to encode taste modality and tastant concentration. Taste bristles present on labellum, legs, wings and ovipositor house dendrites of underlying gustatory receptors neurons (GRNs), which are thus exposed to the environment. On each dendrite different gustatory receptors (GRs) can be co-expressed, that bind different chemicals. GRNs are named according to receptors they express and their induced behaviors. In addition to peripheral taste bristles, GRNs are also located in three clusters that line the pharynx, to monitor food as it enters the esophagus. Taste information is integrated through different mechanisms in primary taste neurons. Adverse tastants can inhibit the activity of appetizing taste neural circuits, as well as the internal state, can modulate the sensitivity of sweet and bitter neurons. GRNs from labellum, pharynx and some of those in the legs project their axons to the sub-esophageal zone (SEZ) of the brain, whereas wing and few leg GRNs project to the thoracic ganglion. The SEZ is a primary gustatory center, with characteristic activation patterns defined based on the origin of the taste information and the type of tastants. Higher brain centers where taste information is conveyed from the SEZ are largely unknown. Second-order sweet projection neurons, relaying taste information from SEZ to the mechanosensory and motor center (AMMC), have been identified. The AMMC is a center for processing of multisensory information, it also receives inputs from olfactory and auditory neurons. Recent work identified taste projection neurons that project to the superior lateral protocerebrum (SLP)

and convey taste information to the mushroom body (MB), in the MB calyx taste inputs continue to be segregated according to the taste modality and origin [84-86].

Olfactory system

D. melanogaster detects odors through the antenna and maxillary palp. These olfactory organs are covered by sensory bristles which house dendrites of underlying olfactory sensory neurons (OSNs). Each OSN generally expresses a single olfactory receptor, belonging to one of the two families of olfactory receptor genes: ORs or IRs, and transmits information to one or two spatially invariant glomeruli in the antennal lobe (AL). In the AL sensory neurons interact with projection neurons that project towards the upper brain centers, and with local neurons whose projections are limited to the AL. Each projection neuron receives information from a single glomerulus and projects its axon to the protocerebrum, and from here to the lateral horn (LH) and the MB. The LH is thought to be important for instinctive olfactory behaviors since premotor neurons receive input in the LH that may be leading to an olfactory behavioral response. MB is important for learning and memory. It receives olfactory, gustatory, and visual input, allowing multimodal processing and memory [87].

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