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Authors
Rengarajan, Sophie
Hallem, Elissa A

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Olfactory circuits and behaviors of nematodes

Sophie Rengarajan and **Elissa A. Hallem**

Department of Microbiology, Immunology, and Molecular Genetics University of California, Los Angeles Los Angeles, California 90095

Sophie Rengarajan: srengarajan@mednet.ucla.edu; Elissa A. Hallem: ehallem@ucla.edu

Summary

Over one billion people worldwide are infected with parasitic nematodes. Many parasitic nematodes actively search for hosts to infect using volatile chemical cues, so understanding the olfactory signals that drive host seeking may elucidate new pathways for preventing infections. The free-living nematode *Caenorhabditis elegans* is a powerful model for parasitic nematodes: because sensory neuroanatomy is conserved across nematode species, an understanding of the microcircuits that mediate olfaction in *C. elegans* may inform studies of olfaction in parasitic nematodes. Here we review circuit mechanisms that allow *C. elegans* to respond to odorants, gases, and pheromones. We also highlight work on the olfactory behaviors of parasitic nematodes that lays the groundwork for future studies of their olfactory microcircuits.

Introduction

Nematodes comprise a large and diverse phylum of roundworms that includes both free-living and parasitic species. Parasitic nematodes of humans, livestock, and plants cause extensive disease and economic loss worldwide. The free-living nematode *C. elegans* has emerged as a model for the study of sensory neurobiology. *C. elegans* offers many advantages as a model system: it has a small and transparent body, making it possible to image neural activity in real time and to use behavior as a readout of circuit function. Its small nervous system consists of 302 neurons in the adult hermaphrodite and 385 in the adult male [1,2]. The connections among these neurons have been mapped, facilitating the identification of functional microcircuits [1–3]. Studies of the *C. elegans* connectome have shown that similar connectivity motifs are found in both *C. elegans* and mammalian cortex [3], suggesting that similar computational units operate across diverse taxa. Recent technical advances have made probing neural circuit function in intact animals feasible with the ability to induce and reversibly manipulate neural activity through genetics, pharmacology, light,

[1]Corresponding author address: Elissa Hallem, UCLA MIMG, 237 BSRB, 615 Charles E. Young Dr. E., Los Angeles, CA 90095, (310) 825-1778, ehallem@ucla.edu.

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and sound [4–7]. These advances have greatly expanded our knowledge of how olfactory microcircuits drive behavior and how these circuits are contextually modulated.

Different nematode species share conserved positional sensory neuroanatomy [8,9], and thus understanding how *C. elegans* microcircuits generate olfactory behaviors may have direct implications for how analogous microcircuits operate in parasitic nematodes. Although the microcircuits underlying olfactory preferences in parasitic nematodes are poorly understood, recent studies have elucidated the divergent olfactory preferences of different parasitic nematode species. Here we review the olfactory behaviors of free-living and parasitic nematodes, and highlight some of the microcircuit computations underlying olfactory behaviors in *C. elegans.*

Olfaction in *C. elegans*

Olfaction is an important sensory modality for *C. elegans*, enabling it to sense food, pathogens, predators, and conspecifics. Proliferating populations of *C. elegans* are found primarily in fallen rotting fruits, where oxygen (O_2) concentrations are low [10]. When environmental conditions are unfavorable or food is scarce, *C. elegans* enters a developmentally arrested, alternative larval stage called the dauer. Dauers disperse into the soil to search for more favorable environments. Dauers are also phoretic, meaning that they associate with insect vectors that can transport them to more favorable environments [10]. These ecological niches inhabited by *C. elegans* inform the olfactory and gas-sensing strategies of the worm. Like other animals, *C. elegans* responds flexibly to odors and gases, modulating its behavior based on both internal and external contexts. The contextual modulation of olfactory behaviors allows worms to make appropriate behavioral decisions in their current environment.

The olfactory circuit of *C. elegans* consists of a small number of highly interconnected neurons, with an average of 3.5 synapses separating sensory neuron input from motor neuron output [2,3,11]. Using this circuit, *C. elegans* can sense and respond to at least 50 odorants [12]. *C. elegans* expands its coding capacity through dynamic modulation of neurons and microcircuits, including the use of neuromodulators and neuropeptides to create extrasynaptic functional connections between neurons [13]. The computations performed by the *C. elegans* olfactory circuit involve fundamental circuit motifs of neural networks and control systems (*e.g.* feedback inhibition and reciprocal inhibition), suggesting that the mechanisms by which *C. elegans* microcircuits functionally process sensory information and drive contextually appropriate behaviors may be conserved in other nervous systems [14].

Organization of the olfactory system—The primarily olfactory organs of *C. elegans* are the bilaterally symmetric amphid sensilla in the head. Eleven chemosensory neurons extend anterior processes with ciliated endings into each amphid sensillum [12]. In contrast to the olfactory sensory neurons of insects and mammals, those of *C. elegans* each express many different olfactory receptors. As in mammals, most of the olfactory receptors are seven transmembrane domain G protein-coupled receptors [12]. Different pairs of olfactory sensory neurons generally drive attraction or avoidance: odor sensing by the AWA and AWC

neurons promotes attraction, whereas odor sensing by the ASH, ADL, and AWB neurons promotes repulsion (Box 1) [12].

Box 1

Summary of functional properties of selected *C. elegans* chemosensory neurons

A non-exhaustive compilation of selected sensory neuron properties and their functional involvement in different microcircuits that are highlighted by this review. The "schematic of activity" depicts a representative shape of calcium activity of each sensory neuron to a particular stimulus that it senses. These traces were based off the following references: AWC [15], AWA [28], ASH [18], ADL [59], BAG [52]. The variable timescales by which these neurons respond to stimuli is not distinguished in our schematic. For BAG neurons, the trace depicts activity from the *C. elegans* adult. For ADL neurons, the male ADL response is shown in green and the hermaphrodite response is shown in black. The functional properties described were based off the following references: AWC [11,12,15,19–23,33], AWA [28,39], ASH [18,39], BAG [22,44,48,50,52–54], ADL [36,59].

The AWA olfactory neurons are "on-cells" that depolarize in the presence of odors, whereas the AWC olfactory neurons are "off-cells" that hyperpolarize in the presence of odors and depolarize upon odor removal [11,15–17]. AWB neurons show both "off" and "on" responses [11,16–18]. Each of these neurons has synaptic connections with other sensory neurons as well as downstream interneurons [3]. Whereas insect and mammalian sensory neurons are generally dedicated to one sensory modality, most *C. elegans* sensory neurons are polymodal as a consequence of the worm's compact and highly interconnected nervous system. For example, the AWC neurons sense odors, temperature, salt, pH, CO_2, and osmotic stress [11,19–22].

A circuit for olfactory attraction—The microcircuit that mediates olfactory attraction via the glutamatergic AWC neurons is the most well-characterized and involves at least three downstream interneurons – AIY, AIA, and AIB [15,23]. In response to the removal of an attractive odorant, AWC inhibits AIY and AIA via glutamate-gated chloride channels, and activates AIB via AMPA-type glutamate receptors. This organization of the olfactory microcircuit into parallel pathways with inverted polarities resembles that of the vertebrate retina, where photoreceptors synapse onto opposing ON and OFF bipolar cells [15]. The temporal dynamics of AWC neuron responses to on/off patterns of olfactory stimuli correspond to the timescales of AWC-mediated odor-evoked behaviors, suggesting that sensory neuron temporal dynamics instruct behavioral dynamics [24].

Navigational strategies for odor responses—To navigate through odor gradients, *C. elegans* primarily uses klinokinesis, also called a biased random walk, to modulate its turning rate and forward locomotion in response to its changing perception of odor concentration [12]. Worms either increase turns and decrease linear forward motion to reorient themselves away from their last (unfavorable) position, or suppress turns and

increase forward motion to continue moving in the same (favorable) direction [12]. Manipulating the activity of first-order interneurons can mimic chemoattraction, suggesting that navigational strategy is determined at the level of the first-order interneurons [6]. By changing the polarity of klinokinesis in response to increasing and decreasing odor gradients, worms can shift their behavior from odor attraction to odor avoidance.

Mechanisms that determine odor valence—A number of mechanisms operate within the olfactory circuit to encode odor valence, *i.e.* whether an odor is attractive or repulsive. One mechanism involves a guanylate cyclase signaling pathway mediated by the receptor guanylate cyclase GCY-28, which acts in AWC sensory neurons to promote attraction to odors that AWC senses. Loss of *gcy-28* switches AWC from a neuron that mediates attraction to one that mediates repulsion [25]. A similar switch from attraction to repulsion occurs in wild-type animals that are exposed to an odor that is initially attractive for a prolonged period in the absence of food [12,25], raising the possibility that *gcy-28* signaling is part of a normal mechanism that flexibly alters odor valence based on environmental context. This study suggests that *C. elegans* olfactory sensory neurons are not irreversibly hard-wired for attraction or repulsion, but may in fact be more flexible in their responses than previously thought.

The valence of an odor stimulus can depend on its concentration. For example, low concentrations of the food-associated odorant isoamyl alcohol are attractive to *C. elegans*, while high concentrations are less attractive or even repulsive [18]. This valence change occurs because different sensory neurons mediate the response at different concentrations. At low concentrations the response is mediated primarily by the AWC olfactory neurons, while at high concentrations the response is mediated primarily by the ASH polymodal avoidance neurons (Box 1). The AWC response is blocked at high concentrations due to synaptic inhibition from other neurons [18]. A similar mechanism operates in the fruit fly *Drosophila melanogaster,* where the behavioral response to apple cider vinegar shifts from attraction at low concentrations to repulsion at high concentrations due to the recruitment of additional glomeruli [26]. In both of these cases, odor valence is determined by which sensory neurons respond to the odor at a given concentration.

The valence of an odor stimulus can also depend on the presence of other sensory stimuli. First-order interneurons can modulate odor valence by integrating information about odor stimuli with information about other sensory stimuli to generate appropriate behaviors. For example, the AIA interneurons have been implicated in multisensory decision-making for behavioral cues with conflicting valences, such as the attractive odorant diacetyl and the aversive stimulus Cu^{2+} [27]. Multisensory decision-making is an important computation for evolutionarily stable nervous systems but occurs much earlier in *C. elegans* microcircuits (*i.e.* within one synapse of sensory input) than those of insects and mammals because the worm nervous system is so small and shallow. However, how first-order interneurons in worms integrate olfactory stimuli with other types of stimuli to drive appropriate behaviors remains poorly understood and is an active area of research.

Mechanisms of gain control and olfactory adaptation—Like other animals, *C. elegans* is capable of maintaining a dynamic range for sensing odors across concentrations

that span several orders of magnitude. One mechanism for this involves rapidly attenuating sensory neuron responses and normalizing first-order interneuron responses [28]. Attenuation of the sensory neuron response prevents saturation, while normalization of the interneuron response results in a relatively concentration-invariant odor representation. The result is a microcircuit specialized for detecting small increases in odor concentration regardless of the absolute odor concentration. Similar mechanisms of odor coding operate in insects and vertebrates, where first-order interneurons in the olfactory circuit show normalized odor responses that encode odor identity regardless of concentration [29,30].

Another mechanism that may contribute to gain control is feedback inhibition from interneurons onto olfactory sensory neurons. For example, neuropeptide signaling between the AWC olfactory neurons and the AIA interneurons creates a feedback loop that promotes adaption to prolonged odor exposure and may also function as a gain control mechanism by dampening responses to strong odor stimuli (Figure 1A) [23]. Thus, both intracellular and circuit-level mechanisms are used to maintain odor responses across concentrations and promote adaption to prolonged odor exposures.

Mechanisms that contribute to behavioral flexibility and variability—Olfactory responses in *C. elegans* are modulated by external and internal context, memory, sex, and life stage [12,16,31,32]. Multiple circuit mechanisms contribute to this behavioral flexibility. One mechanism involves modulation of chemoreceptor expression levels [31,32]. For example, sex, developmental stage, and feeding status alter expression of ODR-10, an odorant receptor in the AWA sensory neurons that detects the food-associated odor diacetyl [31]. Developing larvae of both sexes and starved adults express high levels of ODR-10, allowing them to find and remain in food. In contrast, adult males express low levels of ODR-10, allowing them to forego food in favor of locating mates [31]. By modulating the response properties of its sensory neurons, the worm can prioritize either food finding or mating in a context-appropriate manner.

In addition to showing context-dependent modulation of behavior, *C. elegans* shows stochasticity in its olfactory behavior. This behavioral variability stems at least in part from variability in interneuron responses: while sensory neuron responses are stereotyped, first-order interneuron responses are variable [33]. Interneuron response variability arises from the stochastic activity of multiple regulatory interneurons in the circuit; silencing these interneurons increases the reliability of first-order interneuron responses and reduces behavioral variability [33]. From an ecological perspective, behavioral variability is presumably advantageous at a population level: olfactory stimuli are often unpredictable, and behavioral variability increases the likelihood that at least some members of the population generate an appropriate behavioral response and survive.

Variability also occurs across populations as a result of genetic polymorphisms. For example, polymorphisms in the tyramine receptor TYRA-3, the neuropeptide Y receptor NPR-1, and the globin GLB-5 all cause population differences in foraging behavior and other chemosensory behaviors [32,34–38]. The behavioral differences that result from these polymorphisms demonstrate that the same olfactory circuit can drive a wide range of behaviors.

Interactions between the olfactory circuit and other sensory circuits—Olfactory signals can be integrated with other sensory stimuli to enhance or suppress behavioral responses. For example, pairing food with an attractive odor causes worms to eat more, whereas pairing it with an aversive odor causes worms to eat less [39]. Odors modulate feeding through a mutual inhibition circuit motif that relies on extrasynaptic neuromodulator signaling (Figure 1B). The increased feeding caused by attractive odors requires serotonin release from the NSM neurons. In contrast, the decreased feeding caused by aversive odors requires release of the neuromodulators octopamine and tyramine from the RIC and RIM interneurons. Serotonin and octopamine/tyramine bind receptors on RIC/RIM and NSM, respectively, and reciprocally block release of the other neuromodulator [39]. This reciprocal inhibition motif permits a bistable "winner take all" output from the circuit that either enhances or suppresses eating [39]. As a result, food intake in *C. elegans* is modulated based on the valence of associated olfactory stimuli, as it is in humans.

Olfactory sensory neurons can also participate directly in other sensory circuits to modulate non-olfactory behaviors. For example, in the presence of high salt concentrations one of the two AWC neurons is recruited as an interneuron into the gustatory circuit by the release of neuropeptides from the salt-sensing ASEL neuron and enhances attraction to salt [21]. By both responding to multiple types of stimuli and modulating behavioral responses to non-olfactory stimuli, olfactory neurons participate in multiple functional microcircuits. Dynamic regulation of these microcircuits through neuropeptide signaling expands the coding capacity of the *C. elegans* nervous system and allows the same neurons to be used for multiple functional microcircuits.

Circuits for learned avoidance of pathogenic bacteria—*C. elegans* displays associative olfactory learning: naïve worms that have never ingested the pathogenic bacterium *Pseudomonas aeruginosa* strain PA14 show either mild attraction or no preference for its odor, whereas worms that have ingested PA14 avoid it [17,40]. Learned avoidance of PA14 involves the RIA interneurons and two insulin-like peptides [41]. INS-7 released from the gas-sensing URX neurons increases the RIA response to PA14 and prevents worms from avoiding PA14. Antagonistically, INS-6 release from the ASI chemosensory neurons promotes learning by silencing signaling from URX onto RIA through the inhibition of *ins-7* expression [41]. The ethological contexts in which insulin peptides regulate learning in wild-type animals remain to be determined.

Recently it was discovered that if *C. elegans* are exposed to PA14 early in development, olfactory imprinting occurs: worms form an aversive memory of the pathogenic bacteria that lasts into adulthood [42]. Separate microcircuits create and retrieve the memory, and transfer of the aversive memory from the formation microcircuit to the retrieval microcircuit involves tyraminergic signaling between the two circuits [42]. These examples of learned PA14 avoidance and aversive imprinting demonstrate that *C. elegans* is capable of learning on multiple timescales, and that learning on different timescales involves distinct circuit computations.

Microcircuits for gas-sensing behaviors—In addition to sensing volatile organic compounds, *C. elegans* senses oxygen (O_2) and carbon dioxide (CO_2). The natural habitat of

C. elegans is fallen rotting fruit, where O_2 levels are low [10]. Consistent with this, wild isolates of *C. elegans* prefer low O_2 environments [43]. O_2 is sensed primarily by the dedicated gas-sensing URX, AQR, PQR, and BAG neurons via soluble guanylate cyclases [43–46]. Variation in O_2-evoked behaviors among *C. elegans* strains is due in part to polymorphisms in NPR-1 and GLB-5 [12,34,35]. The downstream circuitry for O_2 response involves multiple interneurons, including RMG, AIY, AIA, AVB, and AVA [36,47]. High O_2 environments are unfavorable and induce a global arousal state that is driven by the URX neurons and translated to other neurons in the circuit via the RMG interneurons [47]. This circuit architecture generates a long-lasting behavioral state in response to aversive high O_2 environments that promotes rapid escape.

CO_2 is a complex cue for *C. elegans* that may signal the presence of predators, conspecifics, or food. Well-fed *C. elegans* adults avoid CO_2 both in the presence and absence of food [48,49]. However, CO_2-evoked behavior is modulated by feeding status, salt, O_2 environment, and temperature [37,38,48–50]. For example, CO_2 response in adults is regulated by O_2 environment through the O_2-sensing URX neurons and NPR-1, such that the level of ambient O_2 determines whether CO_2 is perceived as aversive or neutral [37,38,48–50]. CO_2 response also varies across life stages, with developmentally arrested dauer larvae showing CO_2 attraction (Figure 2A) [51].

The microcircuits underlying CO_2 response are incompletely understood. CO_2 exposure alters the activity of many sensory neurons, although CO_2 chemotaxis appears to be primarily mediated by the BAG and AFD neurons [22,37,38,48,50,52]. The BAG neurons are depolarized primarily by molecular CO_2 rather than bicarbonate or low pH (Box 1) [53], and this response is mediated by the receptor guanylate cyclase GCY-9 [52,53]. The mechanisms of CO_2 detection that operate in AFD and other CO_2-sensing neurons have not been elucidated. The downstream circuitry that mediates CO_2 chemotaxis is poorly understood, but both the RIA and AIA interneurons display CO_2-evoked activity, implicating them in the CO_2 microcircuit [22,38].

CO_2 not only stimulates chemotaxis, but also inhibits egg-laying [22]. The CO_2-induced inhibition of egg laying is mediated in part by the BAG and AWC sensory neurons [22,54]. This circuit presumably functions to prevent deposition of eggs in unfavorable environments. Through extensive modulation of the O_2 and CO_2 microcircuits, and interactions of these circuits with those driving related behaviors such as egg laying, *C. elegans* can efficiently position itself in favorable environments for feeding and reproduction.

Microcircuits for pheromone-sensing behaviors—The *C. elegans* population consists of both hermaphrodites and males, and *C. elegans* males display mating behaviors toward hermaphrodites. The attraction of males to hermaphrodites is an essential aspect of mating behavior, and involves both volatile pheromones of unknown molecular identity [55] and soluble small-molecule pheromones in the ascaroside family that also mediate dauer formation [56,57]. Male attraction to hermaphrodites is driven by a combination of ascarosides that synergistically promote attraction [56]. Different free-living and parasitic species release different blends of ascarosides, and the behavioral responses to ascarosides are species-specific [58].

Detection of ascarosides by *C. elegans* males is mediated by both male-specific and shared sensory neurons: the four male-specific CEM sensory neurons, as well as the shared ASK and ADL sensory neurons, contribute to pheromone response [36,56,59]. The CEM neurons are unusual in that they show stochastic functional heterogeneity in their ascaroside responses both within and between animals, which may contribute to their encoding of ascaroside concentration [60]. The AIA interneurons act downstream of the ASK sensory neurons to mediate ascaroside attraction [36,57].

While males are attracted to ascarosides released by hermaphrodites, other hermaphrodites are repelled. This sexual dimorphism is regulated by a push-pull circuit motif involving the ADL and ASK sensory neurons [59]. In hermaphrodites the ADL neurons promote ascaroside avoidance (Box 1), whereas in males the ADL neuron response is smaller and eclipsed by the ASK neuron response, which antagonizes ADL-mediated avoidance to promote attraction. This push-pull arrangement can generate opposite behavioral responses depending on the balance of activity between the attractive and repulsive arms of the microcircuit [59], thereby enabling sex-specific responses to the same pheromone.

In wild isolates of *C. elegans*, pheromones are not only important for mating but also promote aggregation behavior, in which worms cluster together in the low O_2 environment found at the edges of a bacterial lawn. Aggregation is regulated by both O_2 and pheromone environments [36]. Responses to O_2 and pheromones are coordinated by a hub-and-spoke microcircuit motif. The RMG interneurons form the hub and sensory neurons form the spokes. RMG is connected to the spoke sensory neurons, including the O_2-sensing URX neurons and the pheromone-sensing ASK neurons, by gap junctions. This hub-and-spoke arrangement enables a single interneuron to regulate a complex behavior involving multiple sensory modalities by coordinately modulating the activity of many different sensory neurons [36].

In summary, *C. elegans* has a small nervous system but expands its coding capacity through the use of neuropeptides and neuromodulators that dynamically alter microcircuit function and composition. These neuropeptides and neuromodulators complement the highly interconnected nature of the nervous system and allow neurons to simultaneously participate in multiple orthogonal microcircuits that all coordinately converge on motor neurons to produce contextually appropriate behaviors. Many of the computational mechanisms found in *C. elegans* are likely used by parasitic nematodes in the context of host-seeking behavior, as discussed below.

Olfaction in parasitic nematodes

Human-parasitic nematodes infect over one billion people globally and cause some of the most neglected tropical diseases [61]. These diseases occur predominantly in low-resource settings and result in reduced work productivity and decreased cognitive performance as a result of chronic morbidity [61]. In addition, parasitic nematodes of livestock and plants result in billions of dollars in economic and food losses each year [62]. Many parasitic nematodes have an environmental infective stage, called the infective juvenile (IJ) or infective third-stage larva (L3i) in the case of insect-parasitic and mammalian-parasitic nematodes, that actively searches for hosts to infect using olfaction in combination with

other sensory modalities [9]. A better understanding of olfaction in parasitic nematodes could therefore lead to new strategies for preventing parasitic nematode infections.

A unique aspect of nematode neurobiology is conserved neuroanatomy: electron microscopy studies of anterior sensory anatomy have demonstrated that even distantly related species have approximately the same number of neurons located in roughly the same positions within the body [8,9]. In addition, laser ablation studies have demonstrated that sensory neuron function is often conserved across free-living and parasitic nematode species [9]. For this reason, studies of *C. elegans* olfaction can directly inform studies of olfaction in parasitic worms.

A number of recent technical advances with skin-penetrating nematodes in the genera *Strongyloides* and *Parastrongyloides* promise to greatly facilitate the study of parasitic nematode sensory neurobiology. These include the ability to generate transgenic nematodes by gonadal microinjection and the ability to conduct genome editing using the CRISPR/Cas9 system [63]. In addition, RNAi has been used successfully with some parasitic nematodes [63,64]. These techniques will enable studies of the neurons and circuits underlying the host-seeking behaviors of parasitic nematodes.

Olfactory behaviors of entomopathogenic nematodes—Entomopathogenic nematodes (EPNs) in the genera *Heterorhabditis* and *Steinernema* are parasitic nematodes that infect and kill insects. They are sometimes referred to as “beneficial nematodes” due to their utility for insect biocontrol. EPN-infection of insects is also of interest as a model for harmful parasitic nematodes that infect humans. Like *C. elegans*, EPNs respond to a diverse array of insect odorants, plant odorants, and CO_2 [51,65–71]. Attraction to plant odorants serves to draw EPNs to locations where their insect hosts feed, and in fact some of the plant odorants that attract EPNs are emitted in response to insect damage [71–74].

CO_2 is a strong attractant for EPNs and is used in combination with both insect- and plant-emitted odorants to locate insect hosts (Figure 2A) [51,65,66,71]. Attraction of EPNs to the odors of live insects is greatly reduced or eliminated when CO_2 is chemically removed, suggesting that CO_2 is a critical host cue [65,67]. However, the relative importance of CO_2 versus insect-specific odorants varies for different EPN species and different insect species [65].

The attractive response of EPN IJs to CO_2 resembles that of *C. elegans* dauer larvae (Figure 2A) [51,65]. Parasitic IJs and *C. elegans* dauers are developmentally analogous life stages [75] that may also be behaviorally analogous: whereas IJs seek out hosts to infect, dauers seek out invertebrate carriers [10]. CO_2 attraction by both IJs and dauers may serve the similar purpose of facilitating interactions with insects. CO_2 also stimulates jumping, a specialized host-finding behavior exhibited by some EPN species in which the IJs propel themselves into the air [51,65]. Thus the same chemosensory cue, CO_2, can stimulate both general and species-specific behavioral responses. As in *C. elegans*, the BAG neurons mediate CO_2-evoked behaviors (Figure 2B), indicating that the neural basis of CO_2 response is at least partly conserved across species regardless of whether CO_2 is an attractive or repulsive cue [51].

Olfactory behaviors of plant-parasitic nematodes—Soil-dwelling plant-parasitic nematodes (PPNs) use the general cue CO_2 in combination with plant-specific odorants to specifically target the roots of host plants [70,76,77]. For at least some species, the attractive response to CO_2 may in fact be a response to low pH resulting from dissolved CO_2 rather than to the CO_2 itself [78]. Some of the plant root volatiles that attract EPNs also attract PPNs, suggesting that there is an ecological cost for the plant associated with the production of these volatiles [79].

Plants also release volatiles such as ethylene that modulate attraction of PPNs to their roots [80]. In addition, volatiles from nearby plants can modulate attraction of PPNs to host plants. For example, when intercropped with crown daisy, the tomato plant is protected from parasitism by the root-knot nematode *Meloidogyne incognita* [81]. Crown daisy roots produce lauric acid, which is attractive for PPNs at low concentrations but repulsive at high concentrations [81], reminiscent of the concentration-dependent effects of isoamyl alcohol on *C. elegans* [18]. After attracting *M. incognita* to crown daisy root, lauric acid appears to disrupt chemotaxis behavior and infectivity by downregulating expression of the FMRFamide-related neuropeptide FLP-18 [81]. The intercropping of certain plants may be a nonhazardous alternative to artificial pesticides: intercropping decreases PPN-induced crop damage through the modulation of PPN chemotaxis behavior.

Olfactory behaviors of mammalian-parasitic nematodes—Mammalian-parasitic nematodes also respond to a chemically diverse array of odorants. The olfactory behaviors of the human-parasitic threadworm *Strongyloides stercoralis* are the most well-studied. *Str. stercoralis* infects approximately 100 million people worldwide and leads to chronic gastrointestinal distress; infections can be fatal for immunocompromised individuals [82]. *Str. stercoralis* is a soil-dwelling worm that infects primarily by penetrating the skin of the feet. As such, *Str. stercoralis* IJs are attracted to a number of human skin and sweat odorants [83,84]. For example, *Str. stercoralis* IJs are attracted to urocanic acid, a histidine metabolite found in mammalian skin that is enriched in the skin of the feet [83]. Many of the odorants that attract *Str. stercoralis* are also known mosquito attractants, suggesting that human-parasitic nematodes and mosquitoes may target humans using some of the same olfactory cues [84]. An exception is CO_2, which is attractive for mosquitoes but repulsive for *Str. stercoralis* and other skin-penetrating nematodes (Figure 2A) [84,85]. CO_2 is presumably not an effective long-range host cue for *Str. stercoralis* due to its route of infection since only very low levels of CO_2 are emitted from human skin [84].

The only passively ingested mammalian-parasitic nematode whose olfactory behavior has been characterized in detail is *Haemonchus contortus,* a parasite of ruminants that is a major cause of livestock disease worldwide [86]. *H. contortus* IJs respond robustly to olfactory cues, but unlike skin-penetrating IJs, they are attracted to CO_2 (Figure 2A) [84]. *H. contortus* is also attracted to grass odor [84]. Attraction to CO_2 and grass may serve to direct *H. contortus* IJs toward the mouths of grazing animals, where they are more likely to be ingested.

Olfactory behaviors of the necromenic nematode Pristionchus pacificus—Olfactory behavior has also been studied in *Pristionchus pacificus*, a necromenic species that

associates with beetles [87]. Necromenic nematodes do not kill their hosts, but rather wait for their hosts to die and then propagate on the host cadaver. As such, necromeny is often considered an evolutionary intermediate between free-living and parasitic life styles. *P. pacificus* is attracted to live beetles as well as beetle odorants, beetle pheromone, and plant odorants [87,88]. Olfactory preferences differ among wild *P. pacificus* strains and among closely related *Pristionchus* species, perhaps reflecting differences in their host preferences [87,88]. Natural variation in the responses of different *P. pacificus* strains to beetle pheromone is associated with the cGMP-dependent protein kinase gene *egl-4* [89], raising the possibility that cGMP signaling contributes to host seeking in parasitic nematodes.

Parasite olfactory preferences exhibit context-dependent modulation—As is the case for *C. elegans*, the olfactory preferences of parasitic nematodes are context-dependent and flexible. For example, both EPN IJs and skin-penetrating IJs exhibit temperature-dependent olfactory plasticity: culturing IJs at different temperatures changes their odor preferences [90]. In the case of the EPN *Steinernema carpocapsae*, the response to 80% of the tested odorants changed as a function of their previous cultivation temperature. IJs are long-lived and can survive in the soil through multiple seasons. The volatiles emitted by both animals and plants change seasonally, and thus temperature-dependent modulation of olfactory behavior may enable IJs to locate hosts despite seasonal changes in volatile emissions [90].

Some parasitic nematodes also show age-dependent changes in their olfactory preferences [90]. For example, the EPN *Steinernema scapterisci* is initially repelled by CO_2 but becomes attracted to CO_2 as the IJs age (Figure 2C). This change in CO_2-evoked behavior may reflect a change in host-seeking strategy: CO_2 avoidance by younger IJs may cause them to disperse into the environment in search of new host niches with more available resources (a high cost but potentially high reward behavior), whereas CO_2 attraction by older IJs may cause them to remain in the proximity of existing host niches (a low cost but lower reward behavior) [90].

Odor preferences of parasitic nematodes are shaped by host specificity and mode of infection—A comparison of olfactory behavior across parasitic nematode species revealed that parasite olfactory preferences reflect host specificity and infection strategy rather than genetic relatedness, and that these parasite-specific preferences have evolved multiple times (Figure 3) [84]. For example, the skin-penetrating rat parasites *Str. ratti* and *Nippostrongylus brasiliensis* share similar odor preferences but are not closely related [84]. That odor preferences reflect parasite lifestyle rather than phylogeny suggests that olfaction plays an important role in the ability of parasitic nematodes to find and infect their hosts.

In summary, parasitic nematodes show species-specific olfactory behaviors despite the fact that sensory neuroanatomy is roughly conserved across nematode species [8,9]. Efforts to study olfactory neural circuits in parasitic nematodes are ongoing. Existing knowledge of sensory neuron function is based exclusively on laser ablation studies; the dynamics of sensory neural activity in parasitic nematodes have not been examined. Although the BAG neurons are the only olfactory neurons shown to have conserved function in parasitic and

free-living worms [51], conserved sensory neurons also drive salt chemotaxis, thermotaxis, and changes in developmental stage in *C. elegans* and mammalian-parasitic worms [8,9]. Based on these studies, sensory neuron function appears to be broadly conserved across free-living and parasitic nematodes. In addition, the RIA interneuron plays a role in thermotaxis in both *C. elegans* and *H. contortus* [91], suggesting that interneuron function may be conserved in at least some cases. Nervous system connectivity has not yet been examined in parasitic nematodes. However, a recent study of the *P. pacificus* pharynx found that although *P. pacificus* and *C. elegans* share a set of 20 homologous pharyngeal neurons, the connectivity of these neurons differs in the two species [92]. Thus, behavioral differences among species may arise from a combination of altered connectivity of the nervous system, the actions of neuromodulators and neuropeptides, and species-specific differences in the functional properties of neurons. Future studies of olfactory circuits in parasitic nematodes should clarify the relative contribution of each of these factors to the evolution of olfactory neural circuits and odor-driven behaviors.

Conclusions

Recent studies of olfactory microcircuits in *C. elegans* have elucidated how the worm responds to odorants across a wide range of concentrations, and how these responses are modulated by environmental stimuli, internal behavioral state, and genotype. With new technical advances that enable nearly whole-brain imaging with single-neuron resolution in freely moving *C. elegans* [93–96], it should now be possible to determine how global changes in brain state alter olfactory microcircuits and to clarify the dynamics of how neurons are recruited into or omitted from these microcircuits.

Studies of olfactory behavior in parasitic nematodes have demonstrated how these parasites use olfactory cues to find and infect hosts, with implications for nematode control. Since molecular and genetic tools are now available for some parasitic worms, the microcircuits that drive these behaviors are at the cusp of discovery. Future studies comparing microcircuit function in *C. elegans* and parasitic nematodes should provide insight into how analogous microcircuits operate in free-living versus parasitic species to support parasite-specific olfactory behaviors.

Highlights

- *C. elegans* encodes complex olfactory behaviors with only a small number of neurons.
- Microcircuit motifs that are fundamental across computational systems encode these behaviors.
- Odor preferences of parasitic worms reflect their host ranges and infection mode.
- Olfactory neuron function is at least partly conserved across nematode species.

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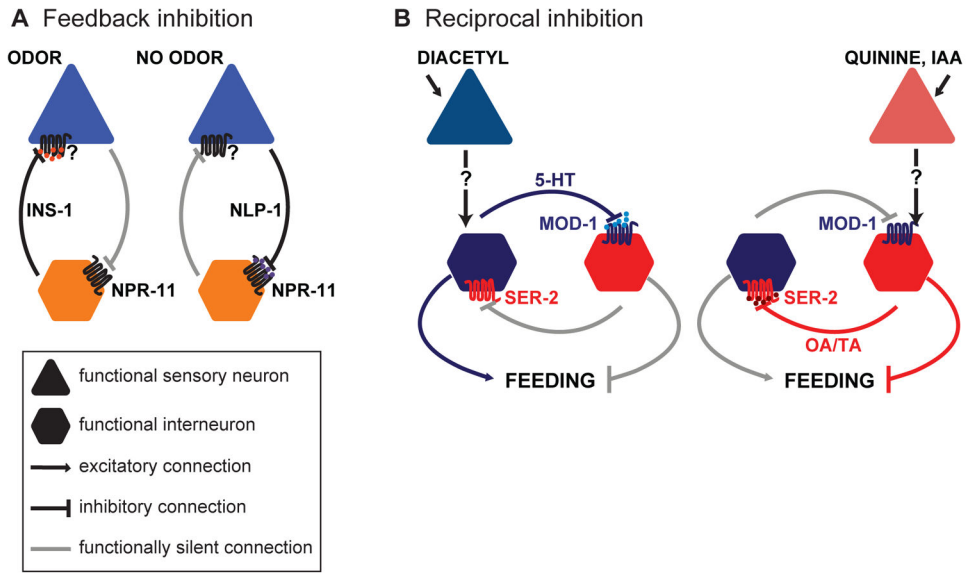


Figure 1. Models of microcircuit motifs present in the *C. elegans* olfactory system

A. A feedback inhibition motif promotes odor adaptation and possibly gain control [23]. The AWC olfactory neurons release NLP-1, which binds NPR-11 on AIA interneurons to inhibit their activity. In the presence of an odor, AWC activity is suppressed. The resulting decrease in NLP-1 signaling permits AIA to release INS-1, which inhibits AWC through an unknown receptor [23]. **B.** Odor environment modulates feeding through a reciprocal inhibition motif [39]. The presence of attractive odors increases feeding, while the presence of repulsive odors decreases feeding. The attractive odorant diacetyl is sensed by the AWA neurons and causes serotonin (5-HT) release from the NSM neurons. 5-HT binds the serotonin-gated chloride channel MOD-1 on the RIM and RIC interneurons, which inhibits them and increases feeding. Repellents such as quinine or high concentrations of isoamyl alcohol (IAA) are sensed by the ASH neurons and promote release of octopamine (OA) and tyramine (TA) from RIM and RIC. OA and TA bind the SER-2 receptor on the NSM neurons and inhibit serotonin release [39].

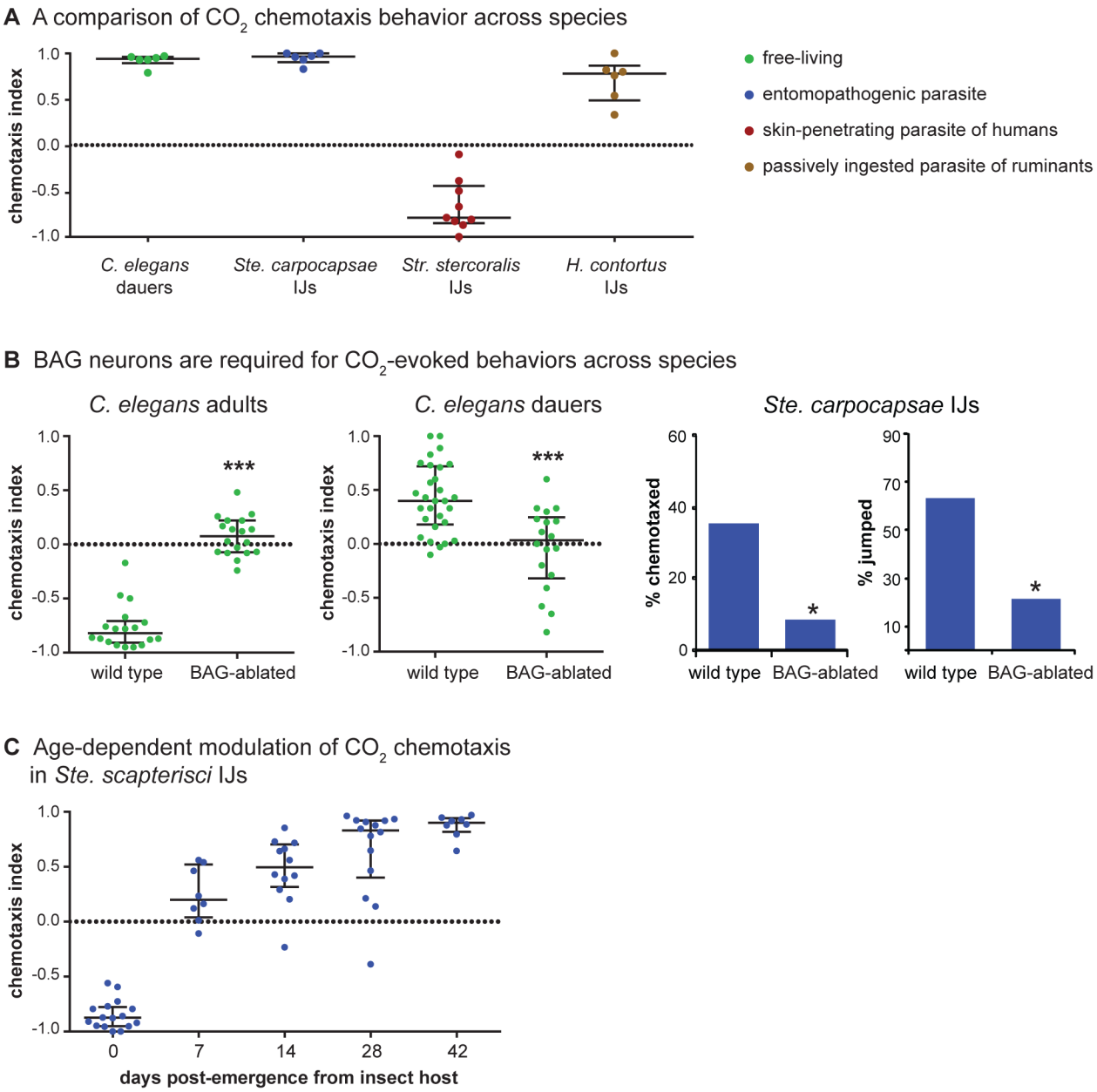


Figure 2. Diverse responses to CO_2 across nematode species

A. CO_2 chemotaxis behavior varies across nematode species [84]. Phoretic *C. elegans* dauers, which seek insect vectors, entomopathogenic *Ste. carpocapsae* IJs, and passively ingested *H. contortus* IJs are attracted to CO_2, while skin-penetrating *Str. stercoralis* IJs are repelled by CO_2 [51,84]. Dauers and IJs were tested in a chemotaxis assay with 10% CO_2, in which the animals were given 1 hr to migrate in a CO_2 gradient. A positive chemotaxis index (CI) indicates attraction and a negative CI indicates repulsion. **B.** The BAG neurons are required for multiple CO_2-evoked behaviors across species. Left, BAG neurons are required for CO_2 chemotaxis in *C. elegans* adults and dauers regardless of whether CO_2 is attractive or repulsive [37,51]. BAG-ablated *C. elegans* adults were tested in a 20 min assay [37], whereas dauers were tested in a 10 min assay [51]. Right, BAG neurons are required

for both CO_2 chemotaxis and CO_2-evoked jumping in *Ste. carpocapsae* IJs [51]. The BAG neurons in IJs were laser-ablated; wild-type animals were mock-ablated. IJs were tested in either a 1 hr chemotaxis assay or a jumping assay in which IJs were given 8 s to jump in response to a 10% CO_2 puff [51]. **C.** The response of *Ste. scapterisci* IJs to CO_2 shifts from repulsion to attraction as the IJs age [90]. IJs were tested in a 1 hr chemotaxis assay with 1% CO_2.

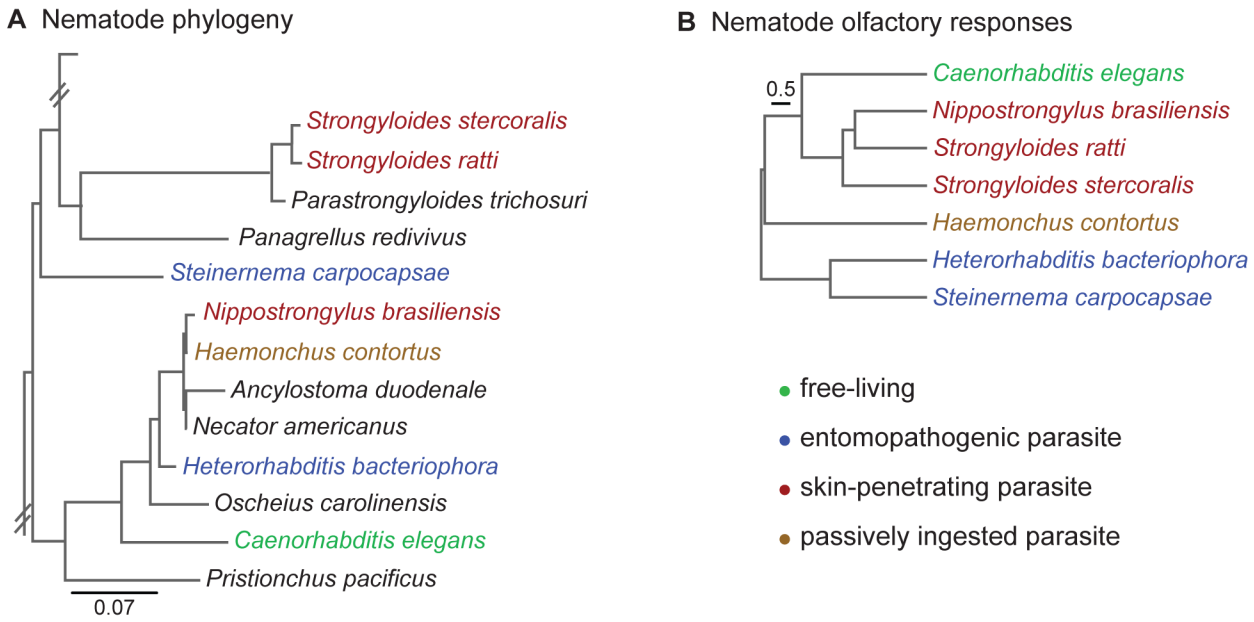
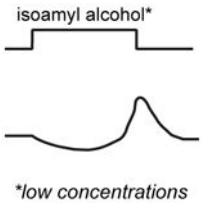
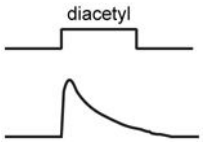
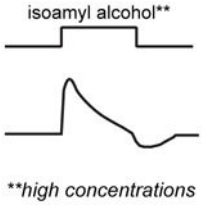
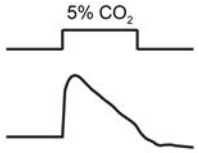
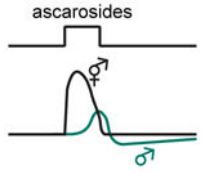


Figure 3. Olfactory responses of parasitic nematodes reflect their host ranges and infection modes rather than their genetic relatedness

A. Schematic of phylogenetic relationships among nematode species [65,84]. Phylogenetic analysis is based on Castelletto *et al.,* 2014 [84] and Dillman *et al.,* 2012 [65]. **B.** A behavioral dendrogram of odor preferences among nematode species [84]. Species cluster based on the hosts they infect and their modes of infection, rather than their genetic relationships. For example, the skin-penetrating rat parasites *Str. ratti* and *N. brasiliensis* show similar odor preferences, even though they are not closely related genetically [84].

Box 1: Functions of selected chemosensory neurons involved in *C. elegans* microcircuits

Neuron:					
Senses:	odors, temperature, CO_2, salt, osmotic stress, pH	odors	odors, soluble chemicals, mechanical and osmotic stimuli	CO_2, O_2	odors, pheromones
Schematic of activity: 					
Valence promoted:	**attraction**	**attraction**	**avoidance**	**avoidance** (adults) **attraction** (dauers)	**avoidance**
Relevant interactions:	• AIB, AIA and AIY (odor attraction) • AIA (feedback inhibition) • RIA (learned odor avoidance) • AIB, RIM and AVA (network variability) • HSN (CO_2 modulation of egg laying)	• AIA (attraction/ gain control) • NSM (reciprocal inhibition circuit)	• RIM/RIC (reciprocal inhibition circuit)	• URX (O_2 modulation of CO_2 response) • HSN (CO_2 modulation of egg laying)	• RMG (hub and spoke circuit) • ASK (push/ pull circuit)

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