Title: Serotonergic modulation of feeding behavior in *Caenorhabditis elegans* and other related nematodes

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Abstract

Serotonin is a conserved neuromodulator that controls feeding behavior in response to environmental inputs in a wide range of species, including the nematode, *Caenorhabditis elegans*. To understand the detailed mechanism and evolution of serotonergic neuromodulation, the feeding behaviors of *C. elegans* and related species have been studied intensively because of their simple neural anatomy and genetic manipulability. *C. elegans* shows patterned movements of a feeding structure called the pharynx, and serotonin modulates feeding rhythms via several serotonin receptors expressed in pharyngeal motor neurons and muscles. Environmental inputs and physiological states like food signals, starvation, and heat affect the activity of serotonergic neurons and downstream neural pathways. We focus on serotonergic neural pathways in the feeding behavior of *C. elegans* and other nematodes, neuromodulation between environmental inputs and behavioral outputs, and their evolutionary path.

Keywords: *tph-1*, serotonin receptor, pharyngeal movement, pharyngeal pumping, peristalsis, environmental inputs, *Pristionchus pacificus*

1 Introduction

Feeding is one of the most fundamental behaviors necessary for animal survival. Serotonin is a conserved neuromodulator regulating feeding behavior in almost all phyla in the animal kingdom (Horvitz et al., 1982; Mathias et al., 1957). Serotonin was first identified in cow serum as a substance that causes contraction of blood vessels (Rapport et al., 1948). Later it was revealed that it plays a role in the nervous system and is involved in various physiological functions and behaviors such as feeding, anxiety, sleep, and learning (Brodie et al., 1955; Mohammad-Zadah et al., 2008; Twarog and Page, 1953). Human pathological studies have shown that disruption of serotonergic activity results in severe disorders, such as depression, schizophrenia, and anxiety disorders, suggesting the importance and complex functions of serotonin (Lin et al., 2014).

The nematode, *Caenorhabditis elegans*, has been utilized as a prominent model organism to investigate neural regulation of feeding behavior because of its transparent body, short life cycle, and simple nervous system (Corsi et al., 2015). Moreover, intensive studies have developed useful tools such as an annotated genome (The *C. elegans* Sequencing Consortium, 1998), forward and reverse genetics (Brenner, 1974; Dickinson et al., 2013; Fire et al., 1998), extrachromosomal arrays to express genes of interest (Mello et al., 1991), connectome maps throughout the body (White et al.,

1986), electropharyngeogram (EPG) (Raizen and Avery, 1994), and optogenetics (Nagel et al., 2005) that facilitate the detailed understanding of molecular mechanisms underlying feeding behavior in *C. elegans*. Serotonin is also used as a neuromodulator in *C. elegans* and is related to several behaviors and physiological functions such as pharyngeal pumping, egg laying, locomotion, mating, and learning (Horvitz et al., 1982; Loer and Kenyon, 1993; Sawin et al., 2000; Zhang et al., 2005).

In addition to usage in neurophysiological studies, *C. elegans* and other related nematodes also provide a model to understand behavioral evolution. To adapt to diverse habitats and food sources, such as bacteria, other nematodes, and fungi, free-living and parasitic nematodes have evolved diverse feeding structures. Even though the mouth forms and feeding styles are different, serotonin is involved in regulation of feeding behaviors in a wide range of nematode species, suggesting the significance of serotonin in the evolution of feeding behaviors in the phylum, Nematoda (Crisford et al., 2018; Komuniecki et al., 2004; Okumura et al., 2017; Weeks et al., 2016; Wilecki et al., 2015).

Here, we review, first, the basic structure and movement of the feeding organ in *C. elegans*; second, the serotonergic nervous systems regulating the feeding behavior; third, the influence of environmental inputs on feeding behavior; and last, studies to understand the feeding behavior of various nematodes.

2 Pharyngeal structure and movements in *C. elegans*

C. elegans feeds on bacteria with a neuromuscular organ called the pharynx, which consists of four parts: the anterior corpus, posterior corpus, isthmus, and terminal bulb (Figure 1A, Albertson and Thomson, 1973). The pharynx in *C. elegans* contains eight classes of muscle cells (pm1-pm8), 14 classes of pharyngeal neurons, marginal cells, epithelial cells, and gland cells.

The movement of the pharynx to swallow food is characterized by two motions of different parts of the organ. One is called pharyngeal pumping, which consists of rhythmic muscle contraction and relaxation in the anterior/posterior corpus, anterior isthmus, and terminal bulb (Figure 1B). Food trapped by pumping in the corpus is crushed by a grinder in the terminal bulb (Doncaster, 1962). The pumping of the corpus and terminal bulb is coordinated via the electrical connection of the pharyngeal muscles with gap junctions (Starich et al., 1996). The other movement is peristalsis in the posterior isthmus. Peristalsis is the contraction of muscles traveling from anterior to posterior (Figure 1B, Avery and Horvitz, 1987). The roles of peristalsis are to concentrate and transport food from the corpus to the terminal bulb. Peristalsis normally occurs every three or four pumps (Avery and Horvitz, 1989).

Recently, a novel pharyngeal movement called “spitting” was reported (Bhatla et al., 2015).

When *C. elegans* senses noxious stimulation, such as UV light via the LITE-1 photoreceptor, pharyngeal pumping immediately stops and then pumping rate increases again; this is called the "burst" response (Bhatla and Horvitz, 2015). Observation of flow direction in worms feeding on mineral oil or beads revealed that during the burst response, the oil or beads trapped into the corpus are expelled rather than retained, indicating that the direction of flow in the pharynx is reversed in response to light (Bhatla et al., 2015). These pharyngeal responses to UV light suggest that the pharynx can control not only the contraction rate but also the direction of flow of the pharyngeal contents (Bhatla et al., 2015).

The pharyngeal nervous system in *C. elegans* is anatomically almost independent of the somatic nervous system. All pharyngeal neurons make synaptic connections within the pharynx except for I1 neurons, a single pair of pharyngeal neurons that receive electrical input from the somatic nervous system through gap junctions (Albertson and Thomson, 1976). Laser ablation of specific pharyngeal neurons has been utilized to investigate how pharyngeal neurons are involved in feeding behavior (Avery and Horvitz, 1989, 1987). For example, MCs are key cholinergic neurons innervating the anterior corpus of the pharynx, which depolarize pharyngeal muscles to increase pumping rate (Figure 2, Raizen et al, 1995), while M3s are glutamatergic neurons that hyperpolarize pharyngeal muscles, causing them to relax (Figure 2, Avery, 1993; Raizen and Avery, 1994; Li *et al.*,

1997). A recent study using optogenetic methods revealed the multiple direct and indirect excitatory pathways controlling pharyngeal pumping. Optogenetic activation of M2 and M4 neurons upregulates pumping, even when MC neurons are ablated. I1 interneurons, which are the only neurons with electrical connections to the somatic nervous system, can stimulate pumping via M2 and MC neurons (Trojanowski et al., 2014). In contrast to complex neural regulation in pharyngeal pumping, isthmus peristalsis requires cholinergic signaling from the M4 pharyngeal neuron; ablation of this neuron completely arrests peristalsis (Figure 2, Avery and Horvitz, 1989).

Despite few synaptic inputs from outside the pharynx, extra-pharyngeal inputs also affect pharyngeal movement in non-synaptic manners. While ablation of all of the pharyngeal neurons or optogenetic inactivation of the pharyngeal cholinergic neurons decrease but do not completely abolish pharyngeal pumping (Avery and Horvitz, 1989; Trojanowski et al., 2014), pan-neuronal inactivation with a chemogenetic method or mutants with defects in acetylcholine release completely cease pharyngeal pumping (Alfonso et al., 1993; Trojanowski et al., 2016). Moreover, serotonergic signaling from extrapharyngeal neurons also has roles in the regulation of pharyngeal movement, as we describe in Chapter 3.

Pharyngeal movement is controlled in a context-dependent manner that is mediated by the serotonin-signaling pathway. Next, we focus on the functions of serotonin neural circuits in the

modulation of feeding behaviors in response to environmental stimuli in *C. elegans*.

3 Serotonergic neural circuit in *C. elegans*

3.1 Serotonergic modulation of feeding rhythms in *C. elegans*

Serotonin is synthesized from an amino acid, tryptophan. Serotonin synthesis is catalyzed by two enzymes, tryptophan hydroxylase (TPH-1) and 5-hydroxytryptophan (5-HTP)/L-dopa decarboxylase (BAS-1), which convert tryptophan to 5-HTP and 5-HTP to serotonin, respectively (Sanders-Bush and Mayer, 1996). This pathway is conserved in *C. elegans*, a wide range of invertebrates including the fruit fly, *Drosophila melanogaster* (Coleman and Neckameyer, 2005), and vertebrates such as humans (Boularand et al., 1995). To understand the functions of serotonin in *C. elegans*, the *tph-1* mutant was generated with reverse genetics by chemical mutagenesis (Sze et al., 2000). *C. elegans tph-1* homozygous mutants are viable, do not produce serotonin, and show abnormal behaviors, including abnormal feeding. In the wild type, the pharyngeal pumping rate increases to more than 200 pumps per minute in the presence of bacterial food from the basal rate of approximately 43 pumps per minute (Avery and Horvitz, 1989). In contrast, the rate of pharyngeal pumping is lower in *tph-1* null mutants than in wild types in the presence of food, but the basal pumping rate is the same, suggesting that serotonin is necessary for upregulation of pharyngeal

pumping in response to food signals but not for basal activity (Sze et al., 2000). Recent analysis over an extended time course showed that serotonin has an inhibitory role in pumping regulation during long-term deprivation from food (Dallière et al., 2016). In wild type worms, pumping rate decreases during the first 120 minutes of food deprivation. After 120 minutes of food deprivation, pumping rate becomes more erratic with individual differences. In *tph-1* mutant, the average pumping rate does not change during the early phase of fasting compared to that of wild types but pumping rate increases only after 4-hour food deprivation, suggesting that serotonin inhibits pumping temporally during prolonged food deprivation (Dallière et al., 2016).

Using immunostaining, serotonin has been identified in the NSM pharyngeal neuron, somatic neurons ADF, AIM, RIG, RIH, VCs, hermaphrodite HSN, male CPs, and male tail neurons (Duerr et al., 1999; Loer and Kenyon, 1993; Sze et al., 2000). The serotonin synthesis enzyme, TPH-1, is expressed only in ADF, NSM, HSN, CPs, and male tail neurons (Sze et al., 2000) and the accumulation of serotonin in AIM and RIH requires the serotonin reuptake transporter MOD-5 (Jafari et al., 2011). Among these neurons, ADF and NSM neurons have functions in modulating pharyngeal pumping depending on the environmental and physiological states (Figure 2, 3). ADF neurons are serotonergic amphid neurons found in the head and are related to chemotaxis and dauer formation (Bargmann and Horvitz, 1991). Several studies emphasize the importance of ADF neurons in

serotonergic upregulation of the pharyngeal pumping rate. Monitoring the neural activity using the calcium indicator GCaMP revealed that ADF neurons are among the sensory neurons that can respond to bacterial food signals (Zaslaver et al., 2015). Cunningham and colleagues showed that expression of TPH-1 in ADF neurons, but not in NSM neurons, in the *tph-1* null mutant rescues the defect of fast pumping in the presence of food. Moreover, ADF-specific knockdown of *tph-1* significantly reduces pumping in response to food (Cunningham et al., 2012). Others demonstrated the contribution of ADF neurons in pumping increment in response to several environmental and physiological changes, which we summarize in detail in Chapter 4 (Gracida et al., 2017; Lemieux et al., 2015; Song et al., 2013). On the other hand, NSM neurons may have functions in feeding regulation in some contexts. NSM neurons are serotonergic neurons in the pharynx (Horvitz et al., 1982; Sze et al., 2000). Ablation of NSM neurons in wild type worms suppressed the duration of pharyngeal fast pumping when the feeding behavior was observed for a long time using a microfluidic device in the presence of food (Lee et al., 2017). In addition, pumping increase induced by attractive odors is mediated by serotonin synthesized in NSM neurons, as we describe in Chapter 4 (Li et al., 2012). A recent study showed that NSM neurons are enteric, serotonergic neurons with their minor neurite extending into the alimentary canal. They detect food ingestion directly via two DEG/ENaC superfamily sodium channels DEL-7 and DEL-3, closely related to acid-sensing ion channels in mammals (Rhoades et al., 2019). The

activation of NSM neurons by food ingestion detected by DEL-7 and DEL-3 induces slower locomotion in response to food, but this type of NSM activation does not alter the pumping rate (Rhoades et al., 2019). These studies suggest that both NSM and ADF neurons are involved in pharyngeal pumping, but ADF neurons have likely major functions in increasing pumping in response to food, and NSM neurons may have subsidiary functions in feeding regulation.

3.2 Serotonin receptors and downstream circuits

Serotonin links environmental and physiological states to pharyngeal movements by modulating the activity of pharyngeal motor neurons, such as MC and M4 neurons, which directly regulate pharyngeal movements (Figure 2). In this section, we focus on downstream neural circuits expressing different serotonin receptors that respond to serotonin signals and modulate the movement of pharyngeal pumping and peristalsis.

There are five serotonin receptors that have been investigated in *C. elegans*: *ser-1*, *ser-4*, *ser-5*, *ser-7*, and *mod-1* (Hamdan et al., 1999; Hapiak et al., 2009; Hobson et al., 2003; Olde and McCombie, 1997; Ranganathan et al., 2000; Tsalik et al., 2003). The former four receptors are 7-transmembrane G-protein-coupled receptors (GPCRs) that were identified using sequence similarities of serotonin receptors in other species (Hamdan et al., 1999; Hapiak et al., 2009; Hobson

et al., 2003; Olde and McCombie, 1997). Pharmacological analyses revealed that these receptors mediate a relatively slow response to serotonin (Hamdan et al., 1999; Hobson et al., 2003; Olde and McCombie, 1997). On the other hand, *mod-1*, which was identified with a forward genetic screening, is a serotonin-gated chloride ion channel that mediates fast response to serotonin by hyperpolarizing neurons (Ranganathan et al., 2000).

Exogenous dose of serotonin induces fast pharyngeal pumping that mimics the response to food signals (Hobson et al., 2006; Lee et al., 2017). This response is mainly mediated by SER-7, since an exogenous dose of serotonin does not induce increased pumping in *ser-7* null mutants (Cunningham et al., 2012; Gomez-Amaro et al., 2015; Hobson et al., 2006; Lee et al., 2017; Song and Avery, 2012). In *C. elegans*, SER-7 has important roles in the regulation of pharyngeal pumping and peristalsis via distinct signaling pathways during feeding behavior (Figure 2). SER-7 is expressed in several neurons in the pharynx, such as MC, M2, M3, and M4 neurons, and vulval muscles (Hobson et al., 2006). Among them, as explained in Chapter 2, MC and M3 pharyngeal neurons depolarize and hyperpolarize pharyngeal muscles, respectively, and M2 and M4 neurons have a milder effect in the stimulation of pumping compared to MC neurons. In MC neurons, SER-7 activates the $G_s\alpha$ signaling pathway and cholinergic signals to stimulate fast pumping in the corpus (Song and Avery, 2012). SER-7 is also expressed in M3 neurons and serotonin is required for the activity of M3 neurons,

implying that SER-7 may also function in M3 neurons to contribute to fast pumping, along with MC neurons (Hobson et al., 2006; Niacaris and Avery, 2003; Trojanowski et al., 2014). Moreover, SER-7 is expressed in the M4 neuron that innervates the posterior isthmus and has an essential function in isthmus peristalsis (Avery and Horvitz, 1987; Avery and Horvitz, 1989). SER-7 in the M4 neuron activates the $G_{12}\alpha$ signaling pathway and dense-core vesicle neurotransmission to stimulate isthmus peristalsis (Song and Avery, 2012). Pharyngeal pumping and peristalsis are coupled but regulated by separate neural pathways, suggesting that serotonin regulates pharyngeal movement in distinct but somehow linked mechanisms (Song and Avery, 2012).

SER-5 is a serotonin receptor, whose physiological functions were examined in *C. elegans* most recently (Hapiak et al., 2009), and an important regulator in feeding rhythms. SER-5 is expressed in body wall muscles, vulval muscles, and head neurons (Carre-pierrat et al., 2006; Hapiak et al., 2009). In *ser-5* mutants, exogenous serotonin does not induce increased pumping (Cunningham et al., 2012). Serotonergic signals from ADF neurons are critical for the upregulation of pharyngeal pumping via the SER-5 serotonin receptor but not the SER-7 nor SER-1 receptors (Cunningham et al., 2012). SER-5 inactivates AAK-2, an AMP-activated kinase (AMPK), which is a conserved regulator of energy balance, through $G_s\alpha$ signaling pathway and protein kinase A (PKA) in neurons expressing the transcriptional factor HLH-34. HLH-34 is an orthologue of the mammalian *single-minded-1*

(*SIM-1)* gene that is required for development of the hypothalamic nucleus associated with energy balance and obesity (Michaud et al., 2001). Serotonergic neurotransmission activates glutamatergic neurotransmission in HLH-34-expressing neurons to upregulate pharyngeal pumping (Figure 2, Cunningham *et al.*, 2012). Although these results suggest that SER-5 responds to serotonin secreted from ADF neurons, other studies show SER-7 mediates serotonergic transmission from ADF neurons in a context-dependent manner, as we discuss in Chapter 4 (Gracida et al., 2017; Song et al., 2013).

SER-1 is not essential for the stimulation of pharyngeal pumping, but it is likely that SER-1 plays a role in the "fine-tuning" of pumping. Treatment with exogenous serotonin increased the pumping rate in both wild types and *ser-1* null mutants (Hobson et al., 2006; Lee et al., 2017) or showed a partially weakened pumping increase in *ser-1* null mutants (Dernovici et al., 2006; Gomez-Amaro et al., 2015). Similarly, two studies have shown that bacterial food stimulates pumping rate in both wild types and *ser-1* null mutants (Dernovici et al., 2006; Hobson et al., 2006), but one study has demonstrated that the increase in pumping in response to bacterial food is partially suppressed in *ser-1* mutants (Lee et al., 2017). Following detailed observation, *ser-1* null mutants were found to exhibit more variance in their pumping rate compared to that of wild types (Hobson et al., 2006), suggesting that the function of SER-1 is stabilization of the pumping rate rather than upregulation. Although there are variations in the expression pattern of *ser-1* depending on the

promoter sequence, SER-1 is expressed in pharyngeal muscles (Tsalik et al., 2003; Xiao et al., 2006), head neurons (Dernovici et al., 2006; Tsalik et al., 2003; Xiao et al., 2006), vulval muscles (Xiao et al., 2006), and tail neurons (Dernovici et al., 2006). The downstream signaling pathway of SER-1 in the regulation of feeding rhythms is unknown; however a PDZ-binding domain in the C-terminus of SER-1 might be functionally important because the PDZ-binding domain has a significant role in egg-laying stimulation (Xiao et al., 2006).

SER-4 and MOD-1 may inhibit neural activity. SER-4 is a GPCR mediated by the $G\alpha_{i/o}$ class of G-proteins to attenuate adenylate cyclase (Olde and McCombie, 1997), and MOD-1 is a serotonin-gated chloride channel, which directly hyperpolarizes cells in response to serotonin (Ranganathan et al., 2000). Both SER-4 and MOD-1 are expressed in head neurons, tail neurons, and ventral nerve cord, but the expression patterns of the two genes do not completely overlap, suggesting that they mediate different neural pathways (Gürel et al., 2012).

The roles of *ser-4* and *mod-1* in pharyngeal movement are controversial. Several studies have shown that *ser-4* mutants can upregulate pumping rate in response to serotonin (Hobson et al., 2006; Srinivasan et al., 2008). However, Song and colleagues revealed that *ser-4* has an inhibitory role in fast pumping (Song et al., 2013), and Lee and colleagues showed that *ser-4* partially enhances the feeding rate in response to food and exogenous serotonin (Lee et al., 2017). Lee and colleagues

utilized a microfluidic recording device to record pharyngeal pumping for much longer periods than most previous studies (Cunningham et al., 2012; Hobson et al., 2006; Lee et al., 2017; Li et al., 2012; Srinivasan et al., 2008). They found that *ser-4* mutants spent less time fast pumping in the presence of food or exogenous serotonin. As for *mod-1*, Lee and colleagues stated the *mod-1* mutant exhibits fast pharyngeal movement like the wild type (Lee et al., 2017), but Song and colleagues showed its inhibitory role in fast pumping (Song et al., 2013), and Li and colleagues revealed that *mod-1* enhances fast pumping in response to exogenous serotonin in the presence of food (Li et al., 2012). The controversial results of *ser-4* and *mod-1* among these studies may be derived from differences in experimental conditions, such as serotonin concentration, developmental stages, and existence of food, indicating that further investigation is necessary to understand the functions of these receptors.

4 Environmental inputs affect feeding rhythms in *C. elegans* through the serotonergic pathway

Several environmental and physiological states are related to feeding modulation through serotonergic neurons and downstream neural pathways (Figure 3).

Food-related sensory cues are environmental inputs that alter the feeding rhythms in *C. elegans* (Figure 3A). Song and colleagues examined the effects of food familiarity on feeding and found that exposure to familiar food enhances pharyngeal pumping (Song et al., 2013). Familiar foods

activate ADF neurons and increase the serotonin production in ADF neurons. In contrast, the pumping rate for novel food is lower than that for familiar food, and the increased pumping for familiar food is cancelled by conditioned media containing supernatant of novel bacteria, implicating that sensory cues from novel bacteria suppress the effect of familiar food. ADF neurons activate downstream MC pharyngeal neurons via the SER-7 serotonin receptor. In *ser-7* null mutants, the pumping rate does not increase in response to familiar food. This defect is partially suppressed by mutations in *ser-4* and *mod-1*, suggesting an inhibitory role of *ser-4* and *mod-1* in feeding increment. Similarly, attractive odors increase pharyngeal pumping via the serotonergic pathway and repellent odors suppress feeding by the tyramine/octopamine signaling (Figure 3A, Li et al., 2012). In this context, NSM neurons are critical in upregulating feeding rhythms in response to attractive odorants such as diacetyl and low concentration of isoamylol. There are also inhibitory neurons, such as RIM tyraminergic and RIC octopaminergic neurons, that suppress fast pumping in response to repellents such as quinine. These inhibitory neurons suppress the activation of NSM neurons, which express the SER-2 tyramine receptor. NSM neurons can stimulate fast pumping by inhibiting the activity of RIM and RIC neurons via the MOD-1 serotonin receptor. Therefore, serotonin and tyramine/octopamine circuits can cross-inhibit each other and integrate two contradictory sensory information to regulate feeding behavior (Li et al., 2012).

Nutritional condition is also important in the regulation of feeding rhythms (Figure 3B). Compared with well-fed worms, starved animals feed more when they are reintroduced to bacteria (Avery and Horvitz, 1990; Lemieux et al., 2015). This hyperactive feeding is mediated by serotonin signaling, and kynurenic acid is an upstream factor of serotonin. Kynurenic acid is derived from tryptophan, and the production of kynurenic acid is catalyzed by NKAT-1. Starvation suppresses the production of kynurenic acid, which functions as an antagonist of glutamatergic neurotransmission through NMDA-gated ionotropic receptors. Deprivation of kynurenic acid through starvation leads to activation of NMDA-receptor expressing neurons (AVA neurons), which initiates neuropeptide Y-like signaling. This neuropeptidergic neurotransmission together with food sensory signaling stimulates serotonergic signaling from ADF. Therefore, when the starved worms re-encounter food, both the food sensory signal to ADF neurons and absence of kynurenic acid, which results in neuropeptidergic transmission from AVA neurons toward ADF neurons, increase serotonin signals from ADF neurons to stimulate hyperactive pharyngeal pumping (Lemieux et al., 2015).

Recent studies revealed that heat stress also alters the pharyngeal pumping rate via serotonergic signaling (Figure 3C, left, Gracida et al., 2017; Tatum et al., 2015). When heat-sensing AFD neurons sense a mild increase in temperature or noxious heat, they indirectly transmit a signal to NSM and ADF serotonergic neurons to increase serotonin release, resulting in increased feeding

rhythms. The downstream neural circuit remains to be elucidated. In this context, serotonin also activates a heat shock transcription factor in distant tissues, including germ line cells, via SER-1, contributing to the cellular response to heat stress (Tatum et al., 2015). ADF serotonergic neurons can also respond to noxious heat stress (Figure 3C, right, Gracida et al., 2017). When worms are exposed to high temperature, pharyngeal pumping decreases, even in the presence of food. After 1-hour recovery at room temperature, the worms increase pharyngeal pumping to a higher level compared with animals before heat stress. This feeding dynamics is mediated by components of the E3 ubiquitin ligase, whose subcellular localization is enriched in the nucleus transiently upon heat stress in ADF neurons. Serotonin signaling transmitted from ADF neurons increases and upregulates pharyngeal pumping via SER-7 and, partially, SER-5. The re-localization of the E3 ligase component also occurs in mutants that have defects in small vesicle and dense core vesicle transmission, suggesting that this process is regulated cell-autonomously in ADF neurons and independently from the canonical thermosensory circuit. The physiological meaning of upregulation of feeding rhythms after noxious heat stress is currently unknown but it may function for energy recovery to repair damages of cells under the noxious heat stress (Gracida et al., 2017).

5 Serotonergic modulation of feeding rhythms in other nematode species

The detailed mechanism of the feeding behavior in *C. elegans*, as mentioned above and in studies of other related nematodes, provides a good model to understand behavioral evolution. The pharyngeal motion of related nematodes, including the Rhabditidae, Diplogastridae, Cephalobidae, Panagrolaimidae, and Teratocephalidae families, differs in their spatial patterns; the parts of the pharynx that pump and where peristalsis occurs vary among these nematodes (Figure 4, Chiang *et al.*, 2006). For example, in nematodes of the Rhabditidae family, like *C. elegans*, the corpus, anterior isthmus, and terminal bulb pump simultaneously, and peristalsis is only seen in the posterior isthmus, whereas, only the corpus pumps and the rest of the parts induce peristalsis in Diplogastridae such as *Pristionchus pacificus*. Laser cell ablation revealed that the M4 pharyngeal neuron regulates posterior pharyngeal movement in all of these families except for Teratocephalidae, which has been examined as an outgroup. This study implies that the components contributing to the regulation of pharyngeal movements are somehow conserved in a relatively wide range of nematodes, although the pharyngeal movements may vary between them.

The roles of serotonin in feeding behavior have been partially revealed in several nematodes, especially in those of the Rhabditidae family. In the Rhabditidae family, serotonin-positive neurons are well characterized by immunostaining against serotonin (Loer and Rivard, 2007; Rivard et al., 2010) (Figure 4). The number of serotonin-positive neurons in the head is different even

between phylogenetically closely related species. Moreover, a serotonin receptor antagonist, mianserin, does not have the same effects in food-related locomotor behavior. Locomotory rate decreases when starved worms re-encounter bacterial food, and this is called enhanced slowing response. In *C. elegans* mianserin reduces enhanced slowing response, but not basal locomotory rate. In contrast, mianserin lowers basal locomotory rate, but not the locomotory rate in enhanced slowing response in *Caenorhabditis* sp. 3. (Rivard et al., 2010) Together with the identification of neuronal cells related to pharyngeal movements, such as M4 neuron (Chiang et al., 2006), the serotonin signaling in those nematodes seems to be partially conserved, but the regulatory pathway and components in serotonergic modulation seem to differ, even in phylogenetically closely related species.

Genetic and pharmacological studies have shown that serotonin regulates an evolutionary novel feeding behavior in the predatory nematode, *Pristionchus pacificus*, a member of the Diplogastridae family. *P. pacificus* is a satellite model for comparative studies of evolutionary biology (Sommer et al., 1996; Sommer and McGaughran, 2013, Sommer et al, 2015). This nematode has advantages in investigating feeding behavior including an annotated genome, forward and reverse genetics, transgenesis, CRISPR/Cas9 gene editing, and a connectome map for all pharyngeal neurons (Bumbarger et al., 2013; Dieterich et al., 2008; Namai and Sugimoto, 2018; Schlager et al., 2009;

Sommer et al., 1996; Witte et al., 2015). The feeding behavior of *P. pacificus* is associated with mouth-form dimorphism, which is influenced by environmental states during larval stages (Bento et al., 2010). Worms with both mouth forms feed on bacteria, but only the worms with a "wider" mouth form (eurystomatous form) can kill and feed on other worms by opening prey cuticles with movable teeth (Figure 5, Bento et al, 2010, Lightfoot et al, 2016). The predators recognize self-progeny to prevent cannibalism via the small peptide SELF-1 (Lightfoot et al., 2019). The function of serotonin in *P. pacificus* was revealed using CRISPR/Cas9-mediated mutants of serotonin synthesis genes and pharmacological analysis (Figure 5). In *P. pacificus*, the loss of serotonin causes a reduction in the pumping rate during bacterial feeding, suggesting that the function of serotonin in bacterial feeding is conserved between *P. pacificus* and *C. elegans* (Okumura et al., 2017). In addition, serotonin is involved in predatory feeding by regulating tooth movement. Exogenous serotonin triggers the predatory type of pharyngeal movement, tooth movement, and a slower pumping rate (Wilecki et al., 2015). While in wild types, the pharyngeal pumping and tooth movement are highly coordinated in one-to-one ratio, *Ppa-tph-1* mutants exhibit disruption of the correct coordination of the pumping and tooth movement, which results in a decrease in predation on other nematodes (Okumura et al., 2017). Further investigation of serotonergic modulation in *P. pacificus* may help understand how serotonin neural circuits have evolved to regulate novel behavior.

The role of serotonin in feeding behavior and components of the serotonergic modulatory pathway is partially known in some non-free-living nematodes, such as in the parasitic nematode, *Ascaris suum*. In *A. suum*, serotonin has been detected using immunostaining in a pair of neurons likely to be homologous to NSM neurons of *C. elegans* (Johnson et al., 1996). Similarly, serotonin is localized in NSM-like neurons in another parasitic nematode, *Haemonchus contortus* (Rao et al., 2011). Treatment with serotonin increases pharyngeal movement in *A. suum* and other related species (Komuniecki et al., 2004; Weeks et al., 2016), suggesting that the role of serotonin in feeding behavior is somehow conserved in phylogenetically distant species. Some of the serotonin receptors in *A. suum* and *H. contortus* have been characterized. In *A. suum*, several splicing variants of the SER-1-like receptor were cloned, and one of the isoforms has an affinity for the serotonin agonist, lysergic acid diethylamide (LSD), when expressed in COS-7 cells (Huang et al., 1999) and HEK293 cells (Huang et al., 2002). Tissue-specific reverse-transcription (RT-) PCR revealed that this serotonin receptor is expressed in pharyngeal muscles, body wall muscles, and nerve cord and/or hypodermis, implying that this receptor may mediate serotonergic stimulation (Huang et al., 2002). In *H. contortus*, the SER-4-like receptor, 5-HT_{1Hc}, interacts with serotonin and several serotonin receptor agonists (Smith et al., 2003). Serotonin also functions in the feeding behavior of plant pathogenic nematodes with a stylet to penetrate host plant roots. Reserpine, an antagonist of the vesicular monoamine

transporter, inhibits movement of the stylet on the pharynx in potato cyst nematodes, *Globodera pallida* (Crisford et al., 2018). Taken together, serotonin is involved in the regulation of feeding behavior in a wide range of nematodes, even though these nematodes have different mouth structures and feeding habits from those of *C. elegans*.

6 Future perspectives

Decades of investigation have revealed several components of serotonergic regulation and their function in feeding behavior in *C. elegans*. Environmental inputs change the physiological states of serotonergic neurons, and serotonin released from those neurons modulates the activity of its target cells. Pharyngeal neurons expressing serotonin receptors alter the movement of pharyngeal muscles and feeding behavior.

We reviewed the mechanisms through which environmental and physiological cues alter the activity of specific types of serotonergic neurons and downstream pathways (Lemieux et al., 2015; Li et al., 2012). However, it is still unknown how multiple environmental inputs and physiological conditions are integrated and modulate serotonergic neural pathways to induce behaviors.

In addition, direct downstream targets of serotonin or NSM and ADF serotonergic neurons are still unclear, because NSM and ADF neurons do not make synaptic connections to most of the

pharyngeal motor neurons that express serotonin receptors and induce pharyngeal movements. One possibility is that uncharacterized serotonin receptors may be related to serotonergic modulation in *C. elegans* because most of the neurons making synaptic connections to NSM and ADF neurons do not express characterized serotonin receptors. These neurons might play a role in feeding behavior via unknown serotonin receptors. Another possibility is the non-synaptic serotonergic transmission that has been observed in vertebrate and invertebrate species (Albertson and Thomson, 1976; De-Miguel and Trueta, 2005; Dernovici et al., 2006; Harris et al., 2011). If the pharyngeal movements are regulated by diffusible serotonin transmission, it is unknown how serotonin secreted from one type of serotonergic neurons but not from others can induce certain pharyngeal movements in certain conditions; for example, Cunningham and colleagues reported that expression of *tph-1* in ADF neurons, but not in NSM neurons, rescue the defect of *tph-1* mutants in feeding regulation (Cunningham et al., 2012). These questions should be addressed to understand the overall roles of serotonin in the modulation of feeding behavior.

Comparative studies between *C. elegans* and other nematodes form a suitable model to understand the evolution of serotonergic modulation in feeding behaviors; however, there is only little knowledge of the regulatory mechanisms of feeding behavior in other nematodes. Unlike in *C. elegans*, there are few to no genetic tools in many species, making it difficult to analyze molecular

components of the serotonergic regulatory pathway. In these circumstances, satellite model organisms, such as *P. pacificus*, are particularly useful in understanding the molecular mechanism of behavioral evolution. Furthermore, microfluidic platforms for electrophysiological recording that do not require genetic tools to monitor neural and muscle activities may facilitate automated screening and the investigation of pharyngeal regulation in both model and non-model nematodes (Weeks et al., 2016). Together, the combination of pharmacological, genetic, and electrophysiological analyses in other nematodes, especially in satellite model organisms, will promote our understanding of the evolution of feeding behaviors and serotonergic modulation.

Because of the simple nervous system of *C. elegans* and numerous studies in the past decades, the feeding behavior of this animal is a prominent model to examine the nature of neuromodulation. Further studies on this system may contribute to studies on neuromodulation in higher animals, offering insights into the neural modulatory circuit mediating environmental input from sensory neurons and outputs to muscle movements.

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References

Albertson, D.G., Thomson, J.N., 1976. The Pharynx of Caenorhabditis elegans. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 275, 299–325. https://doi.org/10.1098/rsta.1892.0001

Alfonso, A., Grundahl, K., Duerr, J.S., Han, H.-P., Rand, J.B., 1993. The Caenorhabditis elegans unc-17 gene: a putative vesicular acetylcholine transporter. Science (80-.). 261, 617–619. https://doi.org/10.1126/science.8342028

Avery, L., 1993. Motor neuron M3 controls pharyngeal muscle relaxation timing in Caenorhabditis elegans. J. Exp. Biol. 175, 283–297. https://doi.org/10.1242/jeb.00433

Avery, L., Horvitz, H.R., 1990. Effects of starvation and neuroactive drugs on feeding in

Caenorhabditis elegans. J. Exp. Zool. 253, 263–270. https://doi.org/10.1002/jez.1402530305

Avery, L., Horvitz, H.R., 1989. Pharyngeal pumping continues after laser killing of the pharyngeal nervous system of C. elegans. Neuron 3, 473–485. https://doi.org/10.1016/0896-6273(89)90206-7

Avery, L., Horvitz, H.R., 1987. A cell that dies during wild-type C. elegans development can function as a neuron in a ced-3 mutant. Cell 51, 1071–1078. https://doi.org/10.1016/0092-8674(87)90593-9

Bargmann, C.I., Horvitz, H.R., 1991. Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in C. elegans. Neuron 7, 729–742. https://doi.org/10.1016/0896-6273(91)90276-6

Bento, G., Ogawa, A., Sommer, R.J., 2010. Co-option of the hormone-signalling module dafachronic acid – DAF-12 in nematode evolution. Nature 466, 494–497. https://doi.org/10.1038/nature09164

Bhatla, N., Droste, R., Sando, S.R., Huang, A., Horvitz, H.R., 2015. Distinct Neural Circuits Control Rhythm Inhibition and Spitting by the Myogenic Pharynx of C. elegans. Curr. Biol. 25, 1–15. https://doi.org/10.1016/j.cub.2015.06.052

Bhatla, N., Horvitz, H.R., 2015. Light and Hydrogen Peroxide Inhibit C . elegans Feeding through Gustatory Receptor Orthologs and Pharyngeal Neurons. Neuron 85, 804–818.

https://doi.org/10.1016/j.neuron.2014.12.061

Boularand, S., Darmon, M.C., Mallet, J., 1995. The human tryptophan hydroxylase gene. J. Biol. Chem. https://doi.org/10.1074/jbc.270.8.3748

Brenner, S., 1974. The Geneticas of Caenorhabditis elegans. Genetics 77, 71–94. https://doi.org/10.1111/j.1749-6632.1999.tb07894.x

Brodie, B., Pletscher, A., Shore, P., 1955. Evidence That Serotonin Has a Role in Brain Function. Science (80-.). 122, 968. https://doi.org/10.1126/science.122.3177.968

Bumbarger, D.J., Riebesell, M., Rödelsperger, C., Sommer, R.J., 2013. System-wide rewiring underlies behavioral differences in predatory and bacterial-feeding nematodes. Cell 152, 109–119. https://doi.org/10.1016/j.cell.2012.12.013

Carre-pierrat, M., Baillie, D., Johnsen, R., Hyde, R., Hart, A., Granger, L., Segalat, L., 2006. Characterization of the Caenorhabditis elegans G protein-coupled serotonin receptors. Invertablate Neurosci. 6, 189–205. https://doi.org/10.1007/s10158-006-0033-z

Chiang, J.-T.A., Steciuk, M., Shtonda, B., Avery, L., 2006. Evolution of pharyngeal behaviors and neuronal functions in free-living soil nematodes. J. Exp. Biol. 209, 1859–1873. https://doi.org/10.1242/jeb.02165

Coleman, C.M., Neckameyer, W.S., 2005. Serotonin synthesis by two distinct enzymes in Drosophila

melanogaster. Arch. Insect Biochem. Physiol. 59, 12–31. https://doi.org/10.1002/arch.20050

Corsi, A.K., Wightman, B., Chalfie, M., 2015. A transparent window into biology: A primer on Caenorhabditis elegans. WormBook 1–31. https://doi.org/10.1534/genetics.115.176099

Crisford, A., Calahorro, F., Ludlow, E., Marvin, J.M.C., Hibbard, J.K., Lilley, C.J., Kearn, J., Keefe, F., Harmer, R., Urwin, P.E., O'connor, V., Holden-Dye, L., 2018. Identification and characterisation of serotonin signalling in the potato cyst nematode Globodera pallida reveals new targets for crop protection. bioRxiv pre-print 1–51. https://doi.org/10.1101/358358

Cunningham, K.A., Hua, Z., Srinivasan, S., Liu, J., Lee, B.H., Edwards, R.H., Ashrafi, K., 2012. AMP-activated kinase links serotonergic signaling to glutamate release for regulation of feeding behavior in C. elegans. Cell Metab. 16, 113–121. https://doi.org/10.1016/j.cmet.2012.05.014

Dallière, N., Bhatla, N., Luedtke, Z., Ma, D.K., Woolman, J., Walker, R.J., Holden-Dye, L., O'Connor, V., 2016. Multiple excitatory and inhibitory neural signals converge to fine-tune Caenorhabditis elegans feeding to food availability. FASEB J. 30, 836–848. https://doi.org/10.1096/fj.15-279257

De-Miguel, F.F., Trueta, C., 2005. Synaptic and extrasynaptic secretion of serotonin. Cell. Mol. Neurobiol. 25, 297–312. https://doi.org/10.1007/s10571-005-3061-z

Dernovici, S., Starc, T., Dent, J.A., Ribeiro, P., 2006. The Serotonin Receptor SER-1 (5HT2ce) Contributes to the Regulation of Locomotion in Caenorhabditis elegans. J. Neurobiol. 1–16.

https://doi.org/10.1002/neu

Dickinson, D.J., Ward, J.D., Reiner, D.J., Goldstein, B., 2013. Engineering the Caenorhabditis elegans genome using Cas9-triggered homologous recombination. Nat. Methods 10, 1028–1034. https://doi.org/10.1038/nmeth.2641

Dieterich, C., Clifton, S.W., Schuster, L.N., Chinwalla, A., Delehaunty, K., Dinkelacker, I., Fulton, L., Fulton, R., Godfrey, J., Minx, P., Mitreva, M., Roeseler, W., Tian, H., Witte, H., Yang, S., Wilson, R.K., Sommer, R.J., 2008. The Pristionchus pacificus genome provides a unique perspective on nematode lifestyle and parasitism. Nat. Genet. 40, 1193–1198. https://doi.org/10.1038/ng.227

Doncaster, C.C., 1962. Nematode feeding mechanisms. 1. observations on rhabditis and pelodera. Nematologica 8, 313–320. https://doi.org/10.1163/187529262X00125

Duerr, J.S., Frisby, D.L., Gaskin, J., Duke, A., Asermely, K., Huddleston, D., Eiden, L.E., Rand, J.B., 1999. The *cat-1* gene of *Caenorhabditis elegans* encodes a vesicular monoamine transporter required for specific monoamine-dependent behaviors. J. Neurosci. 19, 72–84.

Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., Mello, C.C., 1998. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391, 806–811.

Gomez-Amaro, R.L., Valentine, E.R., Carretero, M., Leboeuf, S.E., Rangaraju, S., Broaddus, C.D., Solis, G.M., Williamson, J.R., Petrascheck, M., 2015. Measuring food intake and nutrient

absorption in caenorhabditis elegans. Genetics 200, 443–454. https://doi.org/10.1534/genetics.115.175851

Gracida, X., Dion, M.F., Harris, G., Zhang, Y., Calarco, J.A., 2017. An Elongin-Cullin-SOCS Box Complex Regulates Stress-Induced Serotonergic Neuromodulation. Cell Rep. 21, 3089–3101. https://doi.org/10.1016/j.celrep.2017.11.042

Gürel, G., Gustafson, M.A., Pepper, J.S., Robert Horvitz, H., Koelle, M.R., 2012. Receptors and other signaling proteins required for serotonin control of locomotion in Caenorhabditis elegans. Genetics 192, 1359–1371. https://doi.org/10.1534/genetics.112.142125

Hamdan, F.F., Ungrin, M.D., Abramovitz, M., Ribeiro, P., 1999. Characterization of a novel serotonin receptor from Caenorhabditis elegans: cloning and expression of two splice variants. J. Neurochem. 72, 1372–1383. https://doi.org/10.1046/j.1471-4159.1999.721372.x

Hapiak, V.M., Hobson, R.J., Hughes, L., Smith, K., Harris, G., Condon, C., Komuniecki, P., Komuniecki, R.W., 2009. Dual Excitatory and Inhibitory Serotonergic Inputs Modulate Egg Laying in Caenorhabditis elegans. Genetics 181, 153–163. https://doi.org/10.1534/genetics.108.096891

Harris, G., Korchnak, A., Summers, P., Hapiak, V., Law, W.J., Stein, A.M., Komuniecki, P., Komuniecki, R., 2011. Dissecting the serotonergic food signal stimulating Sensory-Mediated

aversive behavior in C. elegans. PLoS One 6, 1–9. https://doi.org/10.1371/journal.pone.0021897

Hobson, R.J., Geng, J., Gray, A.D., Komuniecki, R.W., 2003. SER-7b, a constitutively active Gαs coupled 5-HT 7-like receptor expressed in the Caenorhabditis elegans M4 pharyngeal motorneuron. J. Neurochem. 87, 22–29. https://doi.org/10.1046/j.1471-4159.2003.01967.x

Hobson, R.J., Hapiak, V.M., Xiao, H., Buehrer, K.L., Komuniecki, P.R., Komuniecki, R.W., 2006. SER-7, a Caenorhabditis elegans 5-HT 7 -like Receptor, Is Essential for the 5-HT Stimulation of Pharyngeal Pumping and Egg Laying. Genetics 172, 159–169. https://doi.org/10.1534/genetics.105.044495

Horvitz, H.R., Chalfie, M., Trent, C., Sulston, J.E., Evans, P.D., 1982. Serotonin and octopamine in the nematode Caenorhabditis elegans. Science (80-.). 216, 1012–1014. https://doi.org/10.1126/science.6805073

Huang, X., Duran, E., Diaz, F., Xiao, H., Messer, W.S., Komuniecki, R., 1999. Alternative-splicing of serotonin receptor isoforms in the pharynx and muscle of the parasitic nematode, Ascaris suum. Mol. Biochem. Parasitol. 101, 95–106. https://doi.org/10.1016/S0166-6851(99)00059-6

Huang, X., Xiao, H., Rex, E.B., Hobson, R.J., Messer, W.S., Komuniecki, P.R., Komuniecki, R.W., 2002. Functional characterization of alternatively spliced 5-HT2 receptor isoforms from the pharynx and muscle of the parasitic nematode, Ascaris suum. J. Neurochem. 83, 249–258.

https://doi.org/10.1046/j.1471-4159.2002.01067.x

Jafari, G., Xie, Y., Kullyev, A., Liang, B., Sze, J.Y., 2011. Regulation of Extrasynaptic 5-HT by Serotonin Reuptake Transporter Function in 5-HT-Absorbing Neurons Underscores Adaptation Behavior in Caenorhabditis elegans. J. Neurosci. 31, 8948–8957. https://doi.org/10.1523/JNEUROSCI.1692-11.2011

Johnson, C.D., Reinitz, C.A., Sithigorngul, P., Stretton, A.O.W., 1996. Neuronal localization of serotonin in the nematode Ascaris suum. J. Comp. Neurol. 367, 352–360. https://doi.org/10.1002/(SICI)1096-9861(19960408)367:3<352::AID-CNE3>3.0.CO;2-4

Komuniecki, R.W., Hobson, R.J., Rex, E.B., Hapiak, V.M., Komuniecki, P.R., 2004. Biogenic amine receptors in parasitic nematodes: What can be learned from Caenorhabditis elegans? Mol. Biochem. Parasitol. 137, 1–11. https://doi.org/10.1016/j.molbiopara.2004.05.010

Lee, K.S., Iwanir, S., Kopito, R.B., Scholz, M., Calarco, J.A., Biron, D., Levine, E., 2017. Serotonin-dependent kinetics of feeding bursts underlie a graded response to food availability in C. elegans. Nat. Commun. 8, 1–11. https://doi.org/10.1038/ncomms14221

Lemieux, G.A., Cunningham, K.A., Lin, L., Mayer, F., Werb, Z., Ashrafi, K., 2015. Kynurenic Acid Is a Nutritional Cue that Enables Behavioral Plasticity. Cell 160, 119–131. https://doi.org/10.1016/j.cell.2014.12.028

Li, H., Avery, L., Denk, W., Hess, G.P., 1997. Identification of chemical synapses in the pharynx of Caenorhabditis elegans. Proc. Natl. Acad. Sci. U. S. A. 94, 5912–5916. https://doi.org/10.1073/pnas.94.11.5912

Li, Z., Li, Y., Yi, Y., Huang, W., Yang, S., Niu, W., Zhang, L., Xu, Z., Qu, A., Wu, Z., Xu, T., 2012. Dissecting a central flip-flop circuit that integrates contradictory sensory cues in C. elegans feeding regulation. Nat. Commun. 3, 1–8. https://doi.org/10.1038/ncomms1780

Lightfoot, J. W., Wilecki, M., Okumura, M., Sommer, R. J. (2016) 'Assaying Predatory Feeding Behaviors in Pristionchus and Other Nematodes', Journal of Visualized Experiments. (115), e54404, doi:10.3791/54404.

Lightfoot, J.W., Wilecki, M., Rödelsperger, C., Moreno, E., Susoy, V., Witte, H., Sommer, R.J., 2019. Small peptide–mediated self-recognition prevents cannibalism in predatory nematodes. Science (80-.). 364, 86–89.

Lin, S., Lee, L., Yang, Y.K., 2014. Serotonin and Mental Disorders : A Concise Review on Molecular Neuroimaging Evidence. Clin. Psychopharmacol. Neurosci. 12, 196–202.

Loer, C.M., Kenyon, C.J., 1993. Serotonin-deficient mutants and male mating behavior in the nematode Caenorhabditis elegans. J. Neurosci. 13, 5407–5417. https://doi.org/10.1038/sj.emboj.7600057

Loer, C.M., Rivard, L., 2007. Evolution of Neuronal Patterning in Free-Living Rhabditid Nematodes I:

Sex-Specific Serotonin-Containing Neurons. J. Comp. Neurol. 502, 736–767. https://doi.org/10.1002/cne

Mathias, A.P., Ross, D.M., Schachter, M., 1957. Identification and Distribution of 5-Hydroxytryptamine in a Sea Anemone. Nature 180, 658–659.

Mello, C.C., Kramer, J.M., Stinchcomb, D., Ambros, V., 1991. Efficient Gene Transfer in C.elegans: Extrachromosomal Maintenance and Integration of Transforming Sequences. EMBO J. 10, 3959–3970.

Michaud, J.L., Boucher, F., Melnyk, A., Gauthier, F., Goshu, E., Levy, E., Mitchell, G.A., Himms-Hagen, J., Chen-Ming, F., 2001. Sim1 haploinsufficiency causes hyperphagia, obesity and reduction of the paraventricular nucleus of the hypothalamus. Hum. Mol. Genet. 10, 1465–1473. https://doi.org/10.1093/hmg/10.14.1465

Mohammad-Zadah, L.F., Moses, L., Gwaltney-Brant, S.M., 2008. Serotonin : a review. J. vet. Pharmacol. Ther. 31, 31, 187–199. https://doi.org/10.1111/j.1365-2885.2008.00944.x.REVIEW

Nagel, G., Brauner, M., Liewald, J.F., Adeishvili, N., Bamberg, E., Gottschalk, A., 2005. Light Activation of Channelrhodopsin-2 in Excitable Cells of Caenorhabditis elegans Triggers Rapid Behavioral Responses. Curr. Biol. 15, 2279–2284. https://doi.org/10.1016/j.cub.2005.11.032

Namai, S., Sugimoto, A., 2018. Transgenesis by microparticle bombardment for live imaging of

fluorescent proteins in Pristionchus pacificus germline and early embryos. Dev. Genes Evol. 228, 75–82. https://doi.org/10.1007/s00427-018-0605-z

Niacaris, T., Avery, L., 2003. Serotonin regulates repolarization of the C . elegans pharyngeal muscle. J. Exp. Biol. 206, 223–231. https://doi.org/10.1242/jeb.00101

Okumura, M., Wilecki, M., Sommer, R.J., 2017. Serotonin Drives Predatory Feeding Behavior via Synchronous Feeding Rhythms in the Nematode Pristionchus pacificus. G3 7, 3745–3755. https://doi.org/10.1534/g3.117.300263

Olde, B., McCombie, W.R., 1997. Molecular cloning and functional expression of a serotonin receptor from Caenorhabditis elegans. J. Mol. Neurosci. 8, 53–62. https://doi.org/10.1007/BF02736863

Raizen, D.M., Avery, L., 1994. Electrical Activity and Behavior in the Pharynx of Caenorhabditis elegans. Neuron 12, 483–495.

Raizen, D.M., Lee, R.Y.N., Avery, L., 1995. Interacting genes required for pharyngeal excitation by motor neuron MC in Caenorhabditis elegans. Genetics 141, 1365–1382.

Ranganathan, R., Cannon, S.C., Horvitz, H.R., 2000. MOD-1 is a serotonin-gated chloride channel that modulates locomotory behaviour in C . elegans. Nature 408, 470–475.

Rao, V.T.S., Forrester, S.G., Keller, K., Prichard, R.K., 2011. Localisation of serotonin and dopamine in Haemonchus contortus. Int. J. Parasitol. 41, 249–254.

https://doi.org/10.1016/j.ijpara.2010.09.002

Rapport, M.M., Green, A.A., Page, I.H., 1948. Crystalline serotonin. Science (80-.). 108, 329–330. https://doi.org/10.1126/science.108.2804.329

Rhoades, J.L., Nelson, J.C., Nwabudike, I., Yu, S.K., McLachlan, I.G., Madan, G.K., Abebe, E., Powers, J.R., Colón-Ramos, D.A., Flavell, S.W., 2019. ASICs Mediate Food Responses in an Enteric Serotonergic Neuron that Controls Foraging Behaviors. Cell 176, 1–13. https://doi.org/10.1016/j.cell.2018.11.023

Rivard, L., Srinivasan, J., Stone, A., Ochoa, S., Sternberg, P.W., Loer, C.M., 2010. A comparison of experience-dependent locomotory behaviors and biogenic amine neurons in nematode relatives of Caenorhabditis elegans. BMC Neurosci. 11, 1–17. https://doi.org/10.1186/1471-2202-11-22

Sanders-Bush, E. & Mayer, S. E. in Goodman & Gilman's Pharmacological Basis of Therapeutics 9th edn (eds Hardman, J. G. et al.) 249-263 (McGraw-Hill, New York, 1996).

Sawin, E.R., Ranganathan, R., Horvitz, H.R., 2000. C . elegans Locomotory Rate Is Modulated by the Environment through a Dopaminergic Pathway and by Experience through a Serotonergic Pathway. Neuron 26, 619–631.

Schlager, B., Wang, X., Braach, G., Sommer, R.J., 2009. Molecular Cloning of a Dominant Roller Mutant and Establishment of DNA-Mediated Transformation in the Nematode Pristionchus

pacificus. Genesis 47, 300–304. https://doi.org/10.1002/dvg.20499

Smith, M.W., Borts, T.L., Emkey, R., Cook, C.A., Wiggins, C.J., Gutierrez, J.A., 2003. Characterization of a novel G-protein coupled receptor from the parasitic nematode H. contortus with high affinity for serotonin. J. Neurochem. 86, 255–266. https://doi.org/10.1046/j.1471-4159.2003.01849.x

Sommer, R.J., Carta, L.K., Kim, S., Sternberg, P.W., 1996. Morphological , genetic and molecular description of Pristionchus pacificus sp . n . (Nematoda : Neodiplogastridae). Fundam. Appl. Nematol. 19, 511–521.

Sommer, R.J., McGaughran, A., 2013. The nematode Pristionchus pacificus as a model system for integrative studies in evolutionary biology. Mol. Ecol. 22, 2380–2393. https://doi.org/10.1111/mec.12286

Sommer, R.J. in Pristionchus pacificus : a nematode model for comparative and evolutionary biology (edited by Ralf J. Sommer) 19 - 41 (Koninklijke Brill NV, Leiden, The Netherlands, 2015)

Song, B., Avery, L., 2012. Serotonin Activates Overall Feeding by Activating Two Separate Neural Pathways in Caenorhabditis elegans. J. Neurosci. 32, 1920–1931. https://doi.org/10.1523/JNEUROSCI.2064-11.2012

Song, B., Faumont, S., Lockery, S., Avery, L., 2013. Recognition of familiar food activates feeding via

an endocrine serotonin signal in Caenorhabditis elegans. Elife 2, 1–27. https://doi.org/10.7554/eLife.00329

Srinivasan, S., Sadegh, L., Elle, I.C., Christensen, A.G.L., Faergeman, N.J., Ashrafi, K., 2008. Serotonin Regulates C. elegans Fat and Feeding through Independent Molecular Mechanisms. Cell Metab. 7, 533–544. https://doi.org/10.1016/j.cmet.2008.04.012

Starich, T.A., Lee, R.Y.N., Panzareua, C., Avery, L., Shaw, J.E., 1996. eat-5 and unc-7 Represent a Multigene Family in Caenorhabditis elegans Involved in Cell-Cell Coupling. J. Cell Biol. 134, 537–548.

Sze, J.Y., Victor, M., Loer, C., Shi, Y., Ruvkun, G., 2000. Food and metabolic signalling defects in a Caenorhabditis elegans serotonin-synthesis mutant. Nature 403, 560–564. https://doi.org/10.1038/35000609

Tatum, M.C., Ooi, F.K., Chikka, M.R., Chauve, L., Martinez-Velazquez, L.A., Steinbusch, H.W.M., Morimoto, R.I., Prahlad, V., 2015. Neuronal serotonin release triggers the heat shock response in C elegans in the absence of temperature increase. Curr. Biol. 25, 163–174. https://doi.org/10.1016/j.cub.2014.11.040

The C. elegans Sequencing Consortium, 1998. Genome Sequence of the Nematode C . elegans : A Platform for Investigating Biology. Science (80-.). 282, 2012–2018.

Trojanowski, N.F., Padovan-merhar, O., Raizen, D.M., Fang-yen, C., 2014. Neural and genetic degeneracy underlies Caenorhabditis elegans feeding behavior. J. Neurophysiol. 112, 951–961. https://doi.org/10.1152/jn.00150.2014

Trojanowski, N.F., Raizen, D.M., Fang-yen, C., 2016. Pharyngeal pumping in Caenorhabditis elegans depends on tonic and phasic signaling from the nervous system. Sci. Rep. 6, 1–10. https://doi.org/10.1038/srep22940

Tsalik, E.L., Niacaris, T., Wenick, A.S., Pau, K., Avery, L., Hobert, O., 2003. LIM homeobox gene-dependent expression of biogenic amine receptors in restricted regions of the C. elegans nervous system. Dev. Biol. 263, 81–102.

Twarog, B.M., Page, I.H., 1953. Serotonin content of some mammalian tissues and urine and a method for its determination. Am. J. Physiol. 175, 157–161. https://doi.org/10.1152/ajplegacy.1953.175.1.157

Van Megen, H., Van Den Elsen, S., Holterman, M., Karssen, G., Mooyman, P., Bongers, T., Holovachov, O., Bakker, J., Helder, J., 2009. A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. Nematology 11, 927–950. https://doi.org/10.1163/156854109X456862

Weeks, J.C., Roberts, W.M., Robinson, K.J., Keaney, M., Vermeire, J.J., Urban, J.F., Lockery, S.R.,

Hawdon, J.M., 2016. Microfluidic platform for electrophysiological recordings from host-stage hookworm and Ascaris suum larvae: A new tool for anthelmintic research. Int. J. Parasitol. Drugs Drug Resist. 6, 314–328. https://doi.org/10.1016/j.ijpddr.2016.08.001

White, J.G., Southgate, E., Thomson, J.N.., Brenner, F.R.., 1986. The structure of the nervous system of the nematode Caenorhabditis elegans. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 314, 1–340.

Wilecki, M., Lightfoot, J.W., Susoy, V., Sommer, R.J., 2015. Predatory feeding behaviour in Pristionchus nematodes is dependent on phenotypic plasticity and induced by serotonin. J. Exp. Biol. 218, 1306–1313. https://doi.org/10.1242/jeb.118620

Witte, H., Moreno, E., Rödelsperger, C., Kim, J., Kim, J., Streit, A., Sommer, R.J., 2015. Gene inactivation using the CRISPR / Cas9 system in the nematode Pristionchus pacificus. Dev. Genes Evol. 1–8. https://doi.org/10.1007/s00427-014-0486-8

Xiao, H., Hapiak, V.M., Smith, K.A., Lin, L., Hobson, R.J., Plenefisch, J., Komuniecki, R., 2006. SER-1, a Caenorhabditis elegans 5-HT2-like receptor, and a multi-PDZdomain containing protein (MPZ-1) interact in vulval muscle to facilitate serotonin-stimulated egg-laying. Dev. Biol. 298, 379–391. https://doi.org/10.1016/j.ydbio.2006.06.044

Zaslaver, A., Liani, I., Ginzburg, S., Yee, L., Sternberg, P.W., 2015. Hierarchical sparse coding in the sensory system of Caenorhabditis elegans. Proc. Natl. Acad. Sci. 112, 1185–1189.

https://doi.org/10.1073/pnas.1504344112

Zhang, Y., Lu, H., Bargmann, C.I., 2005. Pathogenic bacteria induce aversive olfactory learning in Caenorhabditis elegans. Nature 438, 179–184. https://doi.org/10.1038/nature04216

Figure legends

Fig. 1. Pharyngeal movements in *C. elegans*

(A) Schematics of pharyngeal structure of *C. elegans*. The pharynx of *C. elegans* consists of eight subtypes of pharyngeal muscles (pm1-pm8), which are divided into four parts: anterior corpus, posterior corpus, isthmus, and terminal bulb. A grinder is located in the center of the terminal bulb.

(B) Pharyngeal movement of *C. elegans* is characterized by two motions, pumping (upper) and peristalsis (lower). Pumping is observed in the anterior and posterior corpus, the anterior part of the isthmus, and the terminal bulb in *C. elegans*. The figure shows the phase when pharyngeal muscles contract. Peristalsis is observed in the posterior part of the isthmus.

Fig. 2. Serotonergic neural pathway in feeding in *C. elegans*

In the upregulation of feeding, NSM and ADF serotonergic neurons (shown in green) receive environmental and physiological cues and release serotonin (green particle). An increase in serotonin release induced from these neurons activates MC, M3, M4, and probably M2 motor neurons (shown in pink circles). Through the SER-7 serotonin receptor (blue) and $G_s\alpha$ signaling pathway, MC induces phasic action potentials in pharyngeal muscles through cholinergic signals, resulting in fast rhythmic contraction of pharyngeal muscles in pharyngeal pumping. M3, in contrast, transmits glutamatergic inhibitory signals to pharyngeal muscles, to cease muscle contraction in pumping. M2 and M4 also contribute to the upregulation of pharyngeal pumping, while they play only minor roles. M4 contributes to the induction of isthmus peristalsis by transmitting cholinergic signals to the isthmus. Serotonin activates M4 via the SER-7 and $G_{12}\alpha$ signaling pathway. ADF also transmits serotonergic signals to SER-5 (purple) in *hlh-34* expressing cells and those cells provide glutamatergic signaling (orange particles) to pharyngeal neurons. Circle, triangle, and hexagon represent motor neuron, sensory neuron, and interneuron, respectively. Red arrows represent synaptic connections and the black arrow shows non-synaptic transmissions.

Fig. 3. Environmental inputs affecting pharyngeal motions via serotonergic neural pathways

(A) Food-related external signals such as familiar food and attractive odors activate serotonergic neurons and stimulate fast pumping. Familiar food activates ADF serotonergic neurons, and serotonin secreted from these neurons activates MC neurons via the SER-7 serotonin receptor and increases pharyngeal pumping. Novel food signals inhibit ADF activity in unknown manners. Food-related attractive odors, on the other hand, activate NSM neurons and increase serotonergic signaling, resulting in upregulation of pharyngeal pumping. This serotonergic signals also inhibits feeding-inhibitory neurons (RIM and RIC) via serotonin-gated Cl^- channel MOD-1. TA and OA are for tyramine and octopamine, respectively.

(B) Starvation inhibits kynurenic acid production, which plays a role in nutritional cues. Kynurenic acid inhibits neuropeptidergic signaling from the AVA interneuron to ADF serotonergic neurons by antagonizing an NMDA-gated glutamate channel. The neuropeptidergic signal contributes to the activation of serotonergic neurotransmission in ADF neurons, together with food sensory signaling. This serotonergic signal causes hyperactive pumping when starved worms re-entered food.

(C) Heat-related increase of serotonergic signaling and subsequent fast pumping in the canonical thermosensory-dependent (left) or independent manner (right). Left: AFD neurons sense both

moderate temperature increase and acute noxious heat. AFD neurons transmit signals to both ADF and NSM serotonergic neurons to increase serotonin release, which may cause increased pharyngeal pumping. Right: Noxious heat directly induces serotonin release via the ECS-complex-mediated pathway in ADF neurons. When worms are exposed to noxious heat, E3 ubiquitin ligase components (blue diamonds) transiently localize in the nucleus and then pharyngeal pumping stops. After heat stress, these components are re-localized in the cytoplasm, inducing increase of serotonin release. This serotonergic neurotransmission upregulates pharyngeal pumping via SER-7 and partially SER-5 serotonin receptors.

Green figures represent serotonergic neurons. Circle, triangle, and hexagon represent motor neuron, sensory neuron, and interneuron, respectively.

Fig. 4. Phylogenetic relationship of related nematodes and their serotonergic neurons and pharyngeal behavior

The phylogenic tree contains nematodes in Rhabditina (green), Diplogasterina (blue), Cephalobina (brown), Panagrolaimidae (orange), and several outgroup species such as *A. suum* and *T. lirellus* (black). The table shows the number of serotonin-positive cells found by immunostaining and pharyngeal behavior. Red letters in the table are known serotonin-mediated behaviors. The tree is based on data from (Rivard et al., 2010) and (Van Megen et al., 2009), and serotonergic neurons are based on (Chiang et al., 2006; Crisford et al., 2018; Johnson et al., 1996; Rao et al., 2011; Rivard et al., 2010; Weeks et al., 2016; Wilecki et al., 2015)

Fig. 5. Serotonergic regulatory pathway of *Pristionchus pacificus*

P. pacificus displays two different mouth morphs, eurystomatous (wide mouth) and stenostomatous (narrow mouth). Eurystomatous worms (left) display predatory behavior, feeding on other nematodes, along with bacterial feeding, while stenostomatous worms (right) only feed on bacterial food. Several neurons have been identified as serotonergic neurons in *P. pacificus*. NSM and ADF neurons synthesize serotonin catalyzed by Ppa-TPH-1 and Ppa-BAS-1. Serotonin affects both bacterial feeding with fast pumping of the corpus and predatory feeding with coordinated movement of the corpus and teeth. NSM and ADF are critical for this coordination. Red represents the contribution of serotonin to these movements.

Figure 1

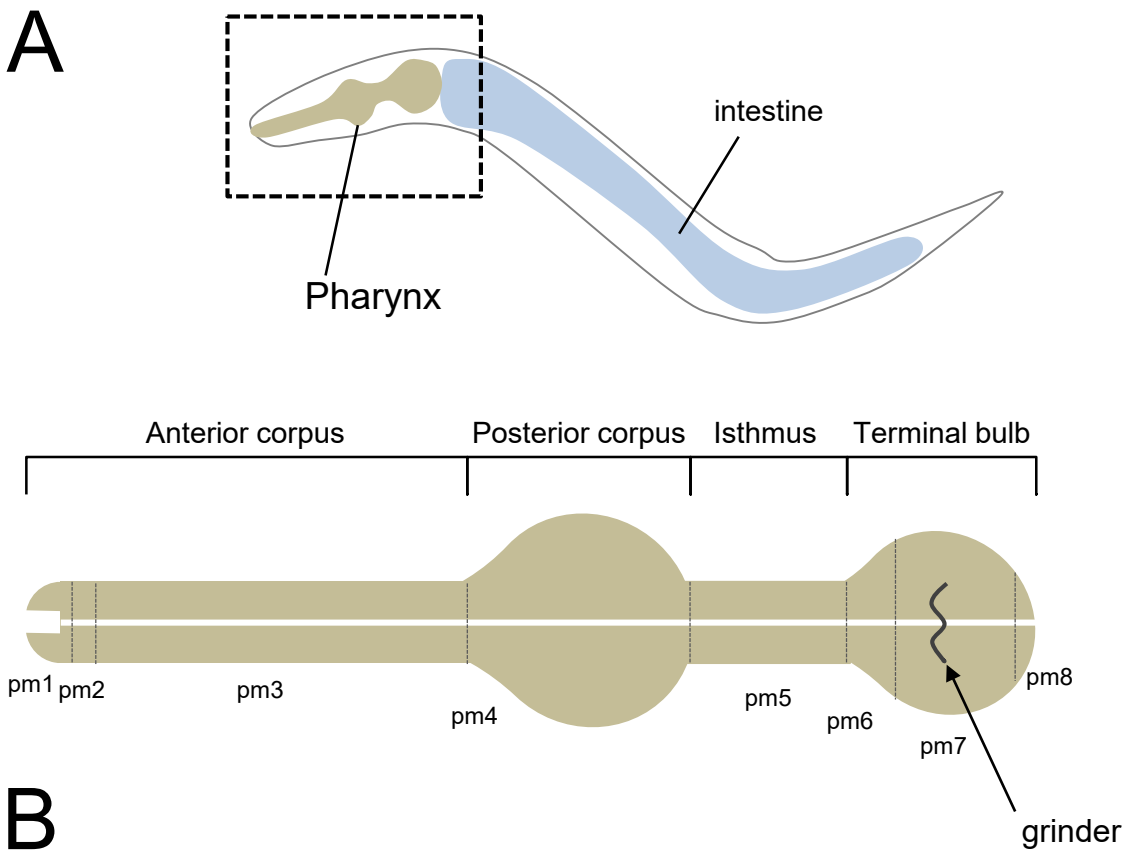

A
intestine
Pharynx
Anterior corpus
Posterior corpus
Isthmus
Terminal bulb
pm1
pm2
pm3
pm4
pm5
pm6
pm7
pm8
grinder
B
Corpus & terminal bulb pumping
Isthmus peristalsis

Figure 2

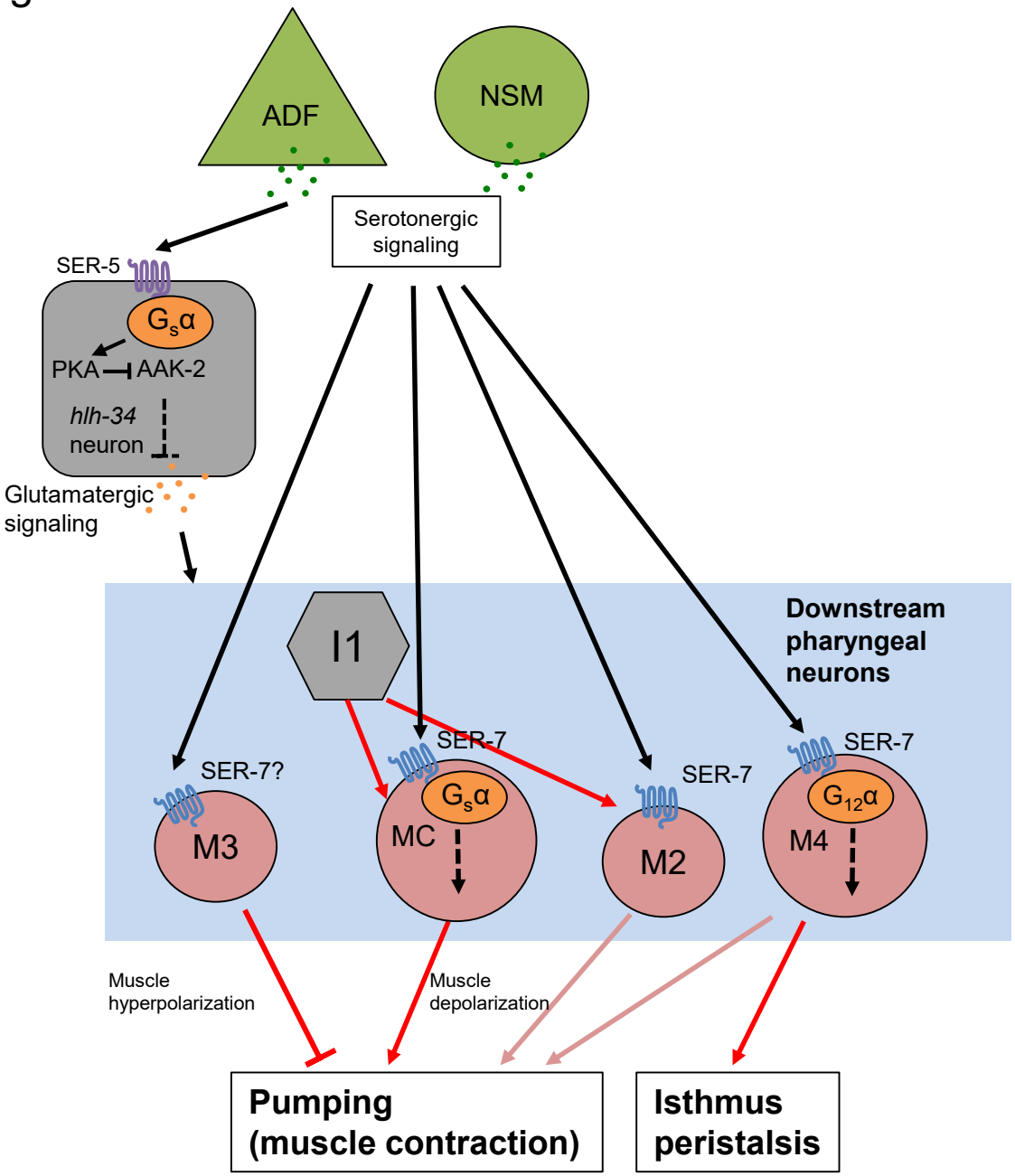

ADF
NSM
Serotonergic signaling
SER-5
$G_s\alpha$
PKA
AAK-2
hlh-34 neuron
Glutamatergic signaling
I1
Downstream pharyngeal neurons
SER-7?
M3
SER-7
$G_s\alpha$
MC
SER-7
M2
SER-7
$G_{12}\alpha$
M4
Muscle hyperpolarization
Muscle depolarization
Pumping (muscle contraction)
Isthmus peristalsis

Figure 3

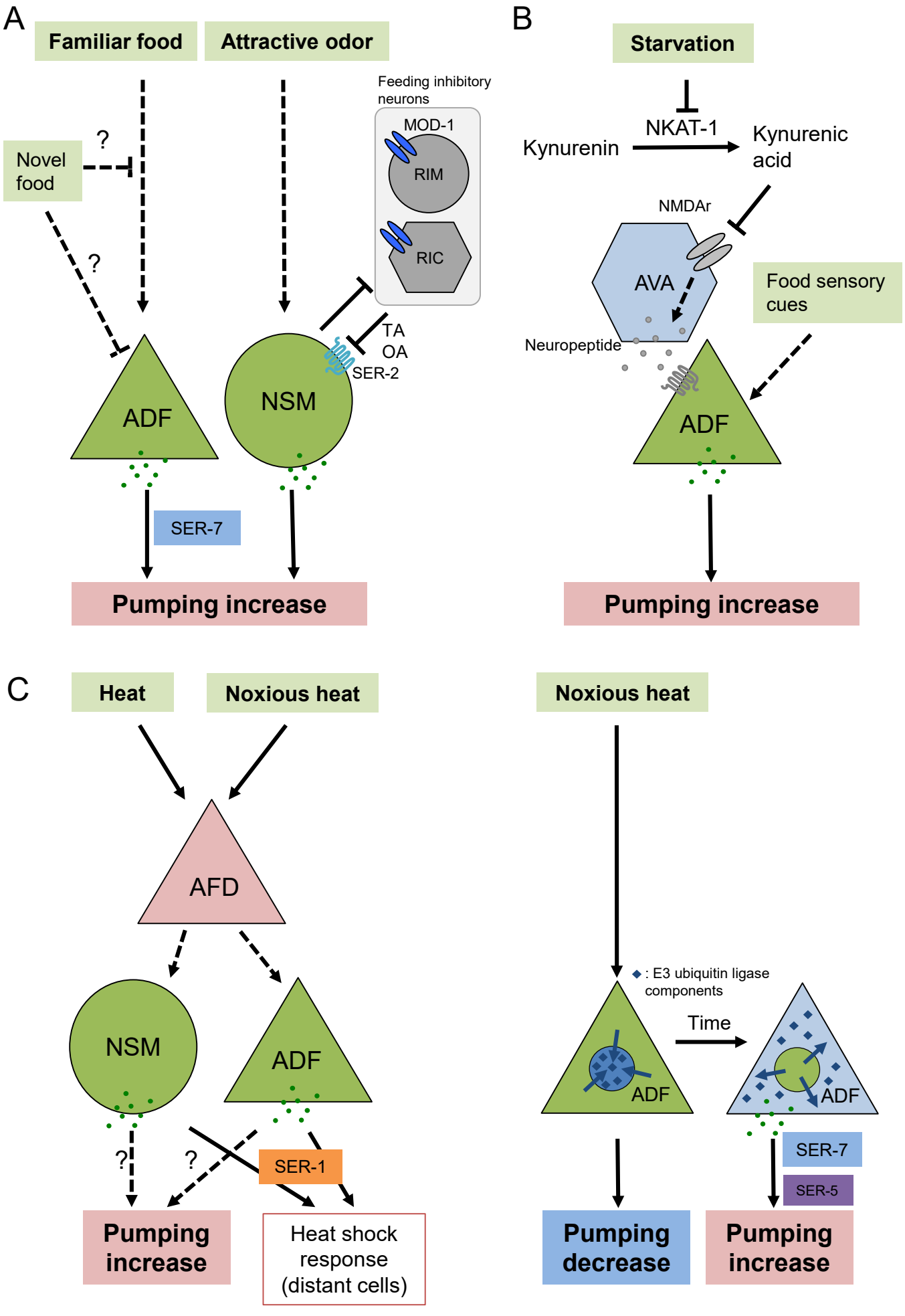
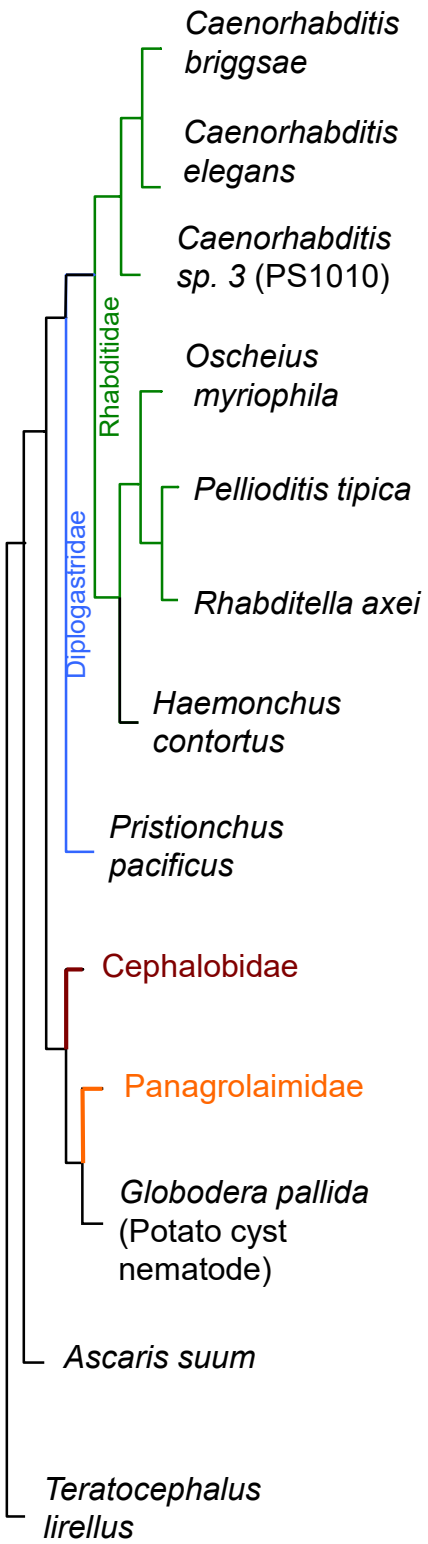

A
Familiar food
Attractive odor
Feeding inhibitory neurons
MOD-1
RIM
RIC
Novel food
?
?
TA
OA
SER-2
ADF
NSM
SER-7
Pumping increase
B
Starvation
NKAT-1
Kynurenin
Kynurenic acid
NMDAr
AVA
Food sensory cues
Neuropeptide
ADF
Pumping increase
C
Heat
Noxious heat
AFD
NSM
ADF
?
?
SER-1
Pumping increase
Heat shock response (distant cells)
Noxious heat
◆ : E3 ubiquitin ligase components
Time
ADF
ADF
SER-7
SER-5
Pumping decrease
Pumping increase

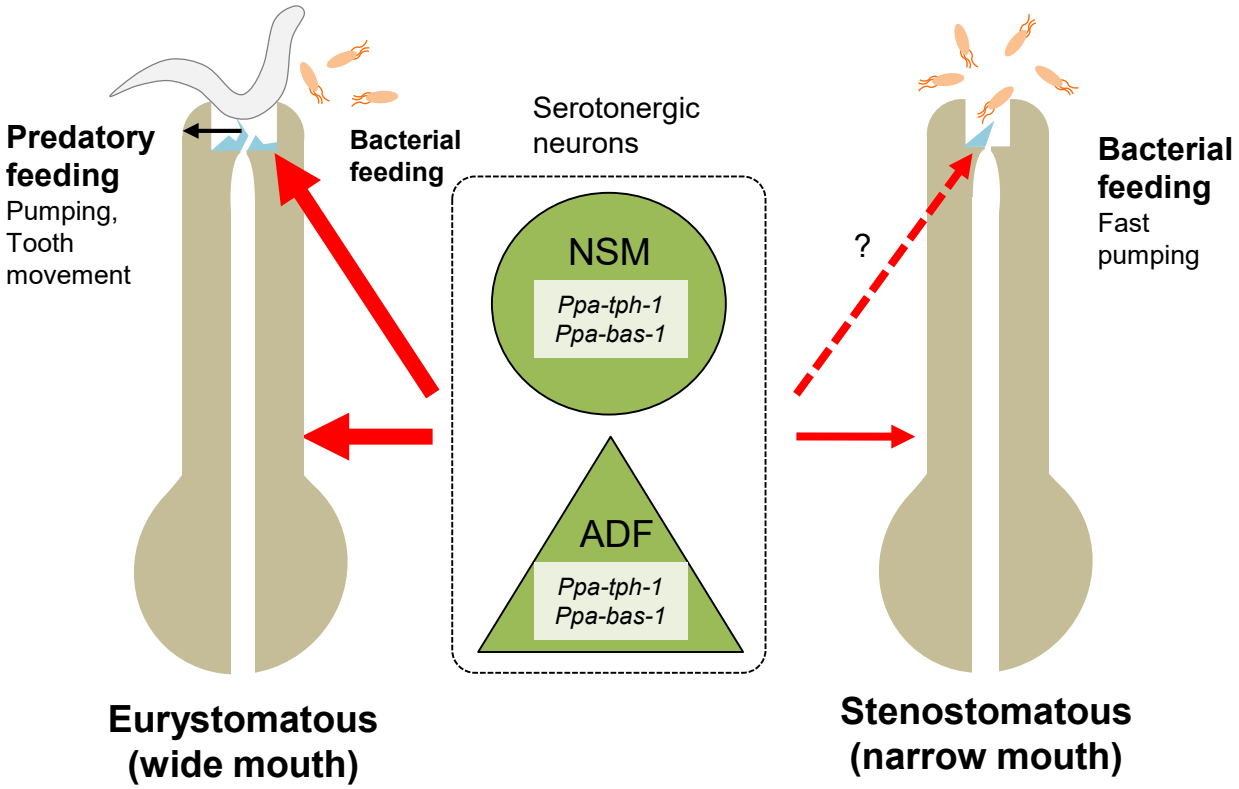
Figure 4



Serotonergic cells in head				Pharyngeal behavior	
NSM	ADF	other	ref.		
+	+	3	Rivard et al, 2010	-	-
+	+	3	Rivard et al, 2010	Corpus/ terminal bulb pumping; posterior isthmus peristalsis	See Figure 1 – 2.
+	+	1 - 3	Rivard et al, 2010	-	-
+	+?	9	Rivard et al, 2010	Corpus/ terminal bulb pumping; posterior isthmus peristalsis	Chiang et al, 2006
+	+?	4 - 5	Rivard et al, 2010	-	-
+	-	2	Rivard et al, 2010	-	-
+?	?	?	Rao et al, 2011	-	-
+	+	2	Wilecki et al, 2015	Corpus pumping; isthmus/ terminal bulb peristalsis; tooth movement	See Figure 5.
?	?	?	-	Corpus pumping; isthmus peristalsis; terminal bulb pumping	Chiang et al, 2006
+	+?	5	Rivard et al, 2010	Corpus pumping; anterior isthmus perisitalsis; terminal bulb pumping	Chiang et al, 2006
+?	+?	?	Chrisford et al, 2018	Stylet ejection (probably via SER-7 and MOD-1)	Chrisford et al, 2018
+?	?	?	Johnson et al, 1996	Pharyngeal pumping and flutter	Weeks et al, 2016
?	?	?	-	Corpus pumping; isthmus peristalsis; terminal bulb pumping	Chiang et al, 2006

Red: serotonin-related behavior

Figure 5


Predatory feeding
Pumping, Tooth movement
Bacterial feeding
Serotonergic neurons
NSM
Ppa-tph-1
Ppa-bas-1
ADF
Ppa-tph-1
Ppa-bas-1
?
Bacterial feeding
Fast pumping
Eurystomatous (wide mouth)
Stenostomatous (narrow mouth)