Chapter 13

Anatomy and connectivity in the pharyngeal nervous system

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Introduction

The nematode pharynx is a pumping organ that transports food from the outside environment to the intestine. It is one of the most prominent and functionally important organ systems in a nematode, and variation in the behaviour and anatomy of the pharynx between species correlates with the tremendous phylogenetic and ecological diversity within the phylum. There are anatomical specialisations for processing various food types, ranging from bacterial grinders in nematodes such as the model organism *Caenorhabditis elegans* to the hypodermic needle-like stomatostylets of many plant-parasitic nematodes. Along with these anatomical specialisations, nematodes vary greatly in details of the behaviour and functioning of the pharynx during feeding. Detailed information pertaining to neural circuits within the pharyngeal nervous system are lacking, greatly limiting the applicability of the comparative method to questions related to the form and function of the nervous system in *C. elegans* and other nematodes.

The cellular level anatomy of the pharynx is best described for *C. elegans*. In this species, as well as most others that have been examined, the pharynx is composed of eight sets of muscle cells, named pm1-pm8 from anterior to posterior (Fig. 13.1). With the exception of the most posterior muscle cell, pm8, all sets of muscle cells exhibit triradiate symmetry, with one dorsal and two ventrosublateral segments.
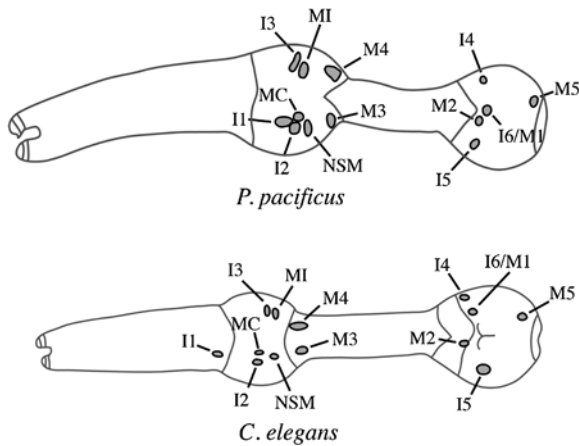
Fig. 13.1. *Positions of neuron nuclei in* Pristionchus pacificus *and* Caenorhab-ditis elegans. *Grey lines outline approximate boundaries of the pharynx and major muscle groups. Black shapes filled with grey indicate the location, size and shape of neuron cell classes. Cell classes with more than one member (I1, I2, M2, M3, MC and NSM) are bilaterally symmetrical. In both species I6 is found on the left hand side of the pharynx and M1 on the right.*

Between these muscle segments are triradiate ventral and superolateral marginal cells. The nematode pharynx is divided into four, functionally specialised, regions, with the first three sets of muscle cells (pm1-pm3) in *C. elegans* making up the procorpus, the next set (pm4) forming the metacorpus, the fifth set of cells (pm5) forming the isthmus and the final three sets of muscle cells (pm6-pm8) forming the terminal bulb. The first two sets of cells in the corpus (pm1, pm2) form part of the mouth opening, or buccal cavity, the rest of the buccal cavity being formed by the tip of pm3 and anterior epidermal cells that are not part of the pharynx. Five gland cells have their nuclei in the terminal bulb of *C. elegans*, two of which open in the terminal bulb, two in the metacorpus and one in the buccal cavity.

A wiring diagram of synaptic connectivity has long been available for the pharyngeal nervous system of *C. elegans* (Albertson & Thomson, 1976). This was, in fact, the first large-scale description of synaptic connectivity based on ultrastructural data and was completed 10 years before the full-animal wiring diagram (White *et al.*, 1986). Recently, a pharyngeal nervous system wiring diagram was completed for a second species, the diplogastrid nematode *Pristionchus pacificus* (Bumbarger

et al., 2013). In both species this nervous system consists of 20 neurons, a nerve ring neuropil in the metacorpus of the pharynx that facilitates communication between neurons. Communication between the pharyngeal and somatic nervous systems appears to be limited to a single pair of gap junctions towards the anterior of the pharynx, as well as through volume transmission *via* biogenic amines such as serotonin.

This chapter will examine the anatomical details of the pharyngeal nervous system of *P. pacificus* within a comparative context. Due to the limited information available, the discussion will focus on comparisons with the model organism *C. elegans*. Further anatomical details about non-neuronal cells of the pharynx in *P. pacificus* will be described elsewhere. For the purpose of this chapter, it is sufficient to mention that *P. pacificus* has the same number and approximately the same arrangement of cell nuclei as *C. elegans*, with the exception that *P. pacificus* lacks two gland cell nuclei present in *C. elegans*.

Overview of the *P. pacificus* nervous system

Although they are not closely related, the general structure of the pharyngeal nervous system is remarkably conserved between *C. elegans* and *P. pacificus* (Bumbarger *et al*., 2013). The relative positions of neuron nuclei are nearly the same (Fig. 13.1), and similarity in the details of neurite position and arborisation within the pharynx makes statements of homology for individual neurons reasonably unambiguous (Chiang *et al*., 2006; Bumbarger *et al*., 2013).

A nerve ring commissure embedded in the metacorpus at the junction between the muscle cells pm4 and pm5 encircles the pharyngeal lumen and serves as the primary point of communication between neurons. In both species, three nerves extend anteriorly from the nerve ring into the procorpus in the dorsal and subventral sectors, and two nerves extend posteriorly into the isthmus in the ventrosublateral sectors (Fig. 13.2). The neurons I6 and M1 enter the posterior nerve ring individually, the former dorsally and the latter dorsally and slightly to the right.

The entry/exit locations of nerves into the nerve ring in *P. pacificus* differ slightly from *C. elegans*, most probably as a result of differences in the anatomy of the interface between pharynx muscles pm4 and pm5. Muscle pm5 forms a larger portion of the median bulb in *C. elegans* than it does in *P. pacificus*. In *C. elegans*, the posteriorly directed
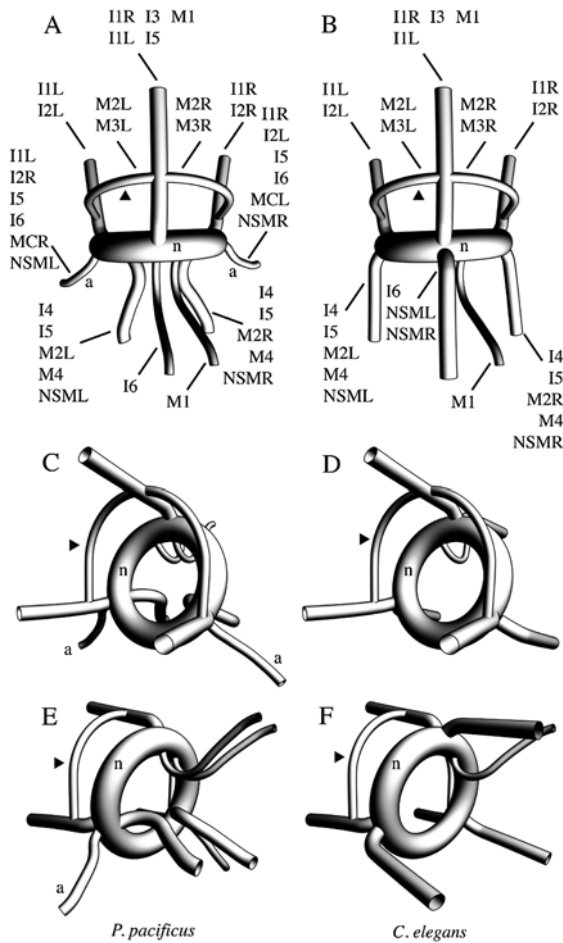
Fig. 13.2. *Comparison of pharyngeal nerve ring structure between* Pristionchus pacificus *and* Caenorhabditis elegans. *A, C, E: The pharyngeal nerve ring of* P. pacificus*; B, D, F: The pharyngeal nerve ring of* C. elegans. *A, B: Dorsal perspective with anterior at the top and posterior at the bottom; C, D: View from anterior and left of the nerve ring: E, F: View from posterior and left of the nerve ring. Capital letters with lines indicate neurons found in each nerve at that location. Black arrowheads indicate the location of the anterior metacorpus commissure. Important differences include the presence of accessory nerves in* P. pacificus*, and medial (*P. pacificus*) vs peripheral (*C. elegans*) connection points for the dorsal and ventrosublateral posterior nerves. Abbreviations: n = pharyngeal nerve ring; a = pharyngeal nerve ring accessory nerve.*

ventrosublateral nerves exit the nerve ring and pass anterior to the M3 cell body as they extend peripherally through the median bulb, then turn to extend posteriorly along the outside of the isthmus. In the ventrosublateral sectors of *P. pacificus*, there are short accessory nerves that extend peripherally anterior to the M3 cell bodies before extending posteriorly (Fig. 13.2), just as the main ventrosublateral nerves do in *C. elegans*, but do not enter the isthmus. These accessory nerves in *P. pacificus* consistently contain processes from I2, I6 and neurosecretory motor (NSM) neurons, and can variably also contain processes from I1, I5 and MC. The neurites originating from the cell bodies of M3 neurons enter the nerve ring at the same location as the accessory nerves.

The relative positions of neurons within the nerve ring have previously been examined only in transverse sections through the animal. This orientation is not ideal, as interpretation will differ according to where in the nerve the section is observed. In order to examine neuron positions in a more relevant way, we constructed 3D models of the nerve ring for both species and used these to examine virtual ventral-radial and subdorsal-radial sections through the nerve ring (Fig. 13.3). These virtual slices reveal some amount of conservation in the placement of neurons within the nerve ring. For example, M4 is conserved in having a medial position in both the ventral and subdorsal regions of the nerve ring. MI is anterior in the ventral nerve ring of both species.

Due to evolutionary changes in neurite morphology, the neurites present in each region of the nerve ring differ between species. In *P. pacificus*, the neurons I4, M4 and MI cross the ventral midline of the nerve ring. In *C. elegans*, two additional neuron classes consistently cross ventrally (NSM and I5). Two others (I2 and I1) vary between individuals in whether they cross or not. NSM in *P. pacificus* is highly unusual in that it is the only pharynx neuron that does not send a process oriented circumferentially within the nerve ring or the anterior metacorpus commissure. It passes through the nerve ring with a longitudinal orientation and does not form synapses at this location; nor does it cross the ventral midline as it does in *C. elegans*. Processes from the neurons I3, I4 and I6 are consistently found in the subdorsal nerve ring of *P. pacificus* but not in *C. elegans*.

A second, smaller, commissure composed of the neuron classes M2 and M3, here termed the anterior metacorpus commissure, bridges each ventrosublateral sector by encircling the pharynx dorsally just anterior to
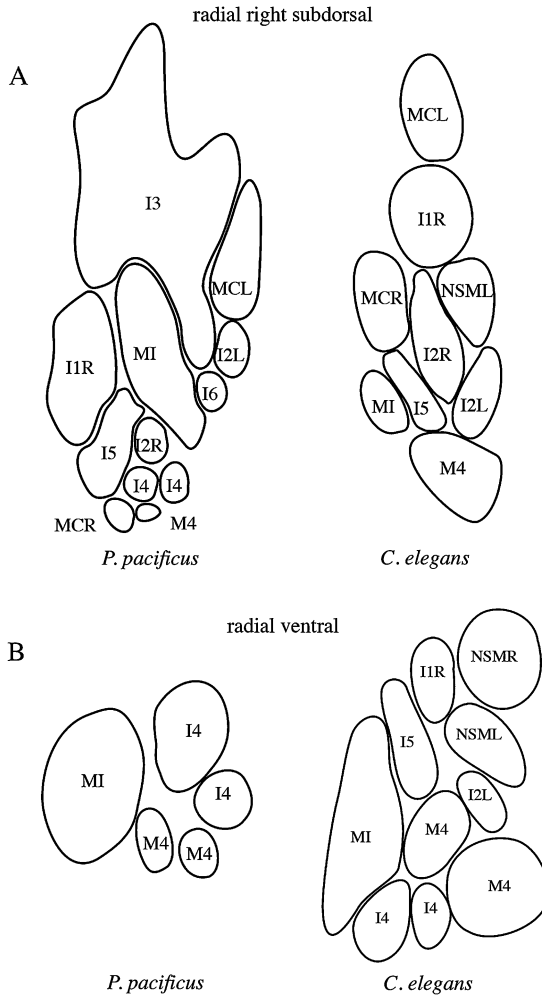
radial right subdorsal

A

I3

MCL

I1R

MI

I2L

I6

MCL

I1R

MCR   NSML

I2R

MI   I5   I2L

I5   I2R

I4   I4

MCR   M4

M4

P. pacificus   *C. elegans*

radial ventral

B

I4

NSMR

I1R

MI

I4

NSML

M4   M4

I5

MI

I2L

M4

M4

I4   I4

P. pacificus   *C. elegans*

Fig. 13.3. *Virtual radial sections through the pharyngeal nerve rings of* Pristionchus pacificus *and* Caenorhabditis elegans. *A: Virtual radial section through the right subdorsal nerve ring; B: Virtual radial section through the ventral nerve ring. In both panels, slices are oriented with anterior to the left, peripheral above, and medial below.*

the nerve ring. In both species this commissure passes through the pm4 muscle cell. In *P. pacificus* it travels between the mc1 and mc2 marginal epithelial cells, whereas our observations indicate that in *C. elegans* it travels through the mc1 cell and does not come in contact with mc2.

A terminal bulb commissure that bridges the ventrosublateral and dorsal regions of the pharynx has been described in *C. elegans* as consisting of processes from I4, I5 and M5. In *P. pacificus*, this commissure is formed primarily by I4 and I5 and appears to be in a similar location. The single process of M5 in *P. pacificus* travels only briefly through the commissure before extending anteriorly with a neurite from I6 into the isthmus. The exact location of the commissure is not described for *C. elegans* but in *P. pacificus* it is located in between the pm5 and pm6 muscle cells and passes through the g1 dorsal gland cell.

The two species appear to have nervous systems constructed of the same component parts with similar anatomy, yet there are significant differences in pharynx function and behaviour that raise questions about structure-function relationships in nervous systems. The regions of the pharynx that are coupled in their activity differ between major groups of nematodes. The metacorpus and terminal bulb are coupled in *C. elegans*, whereas the isthmus and terminal bulb are coupled in *P. pacificus* (Chiang *et al.*, 2006). The terminal bulb of *P. pacificus* and other diplogastrid nematodes has undergone an evolutionary shift from exhibiting pumping activity to being a peristaltic region (Chiang *et al.*, 2006). Perhaps most interestingly, the anterior region of the pharynx in *P. pacificus* has evolved more complex behaviour in order to accommodate multimodal feeding. While feeding on bacteria, the anterior pharynx of *P. pacificus* appears to function very similarly to *C. elegans* and other nematodes, but when predatory on other nematodes a specialised tooth in the mouth opening is actuated and the pumping rate decreases substantially.

Sensory input in the pharynx

Little is known, even for *C. elegans*, about sensory input within the pharyngeal nervous system. No ciliated neurons have been reported within the pharynx of either *C. elegans* or *P. pacificus*. Neurons are designated as putative mechanosensory or proprioceptive neurons when a branch terminates in a subcuticular ending with an attachment to the cuticle lining the pharynx lumen. In *C. elegans*, the neuron classes M3, I1, I2, I3, I5 and I6 contain such subcuticular endings. With the exception of I5, the same neurons have subcuticular endings in *P. pacificus*,

indicating the likelihood that sensory inputs into the pharynx are largely conserved.

The lack of a subcuticular ending in the I5 neuron of *P. pacificus* does not completely rule out a sensory function. I5 in this species forms a ring around the pharynx lumen at the junction between pm4 and pm5, travelling through the terminal bulb commissure on the dorsal side. Here I5 is unusual in that it displays numerous varicosities not correlated with the presence of synapse locations. These varicosities are not present in the equivalent region of I5 in *C. elegans* and could be indicative of specialised function.

M3 was erroneously reported to have two subcuticular endings in *P. pacificus* (Bumbarger *et al.*, 2013). There is a posteriorly directed process but it lacks the darkly staining junctions reported for other putative sensory endings. In *C. elegans*, the M3 putative sensory ending contacts the cuticle of the pharyngeal lumen just posterior to the nerve ring. In *P. pacificus*, however, the sensory ending is associated instead with the cuticle of the subventral gland cell ducts rather than the pharyngeal lumen and forms junctions with the pm4 and pm5 muscle cells.

I1, I2, I3 all have subcuticular endings in similar locations in both species. I1 and I3 attach to the cuticle in between the pm1 and pm2 muscle cells. I2 attaches adjacent to pm1, just anterior to I1; in *P. pacificus*, but not *C. elegans*, it differs in morphology from other pharynx subcuticular endings in that it penetrates into the cuticle, resulting in less of a barrier between it and the outside environment (Fig. 13.4). All
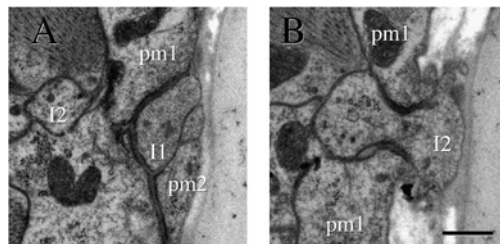

Fig. 13.4. *Subcuticular endings for the neuron cell classes I1 and I2 in* Pristionchus pacificus. *A: I1 projects between muscle cells pm1 and pm2 and terminates adjacent to the cuticle; B: I2 projects through a small pocket formed by the folding on the anterior side of pm1 and terminates within the cuticle, rather than adjacent as for other subcuticular endings in the pharynx. This terminus is immediately anterior to that of I1. (Scale bar = 0.5 μm.)*

three of these neuron classes in *P. pacificus* were observed to contain an accumulation of vesicles close to the ending, although no presynaptic densities are nearby.

In *C. elegans*, the subcuticular ending of I6 is found at the distal end of a neurite that originates at the posterior neuron cell body and attaches to the cuticle between muscle cells pm5 and pm6. In *P. pacificus* the equivalent neurite has a more complex morphology, described in more detail in the individual neuron descriptions below. The cuticle attachment point of I6 in *P. pacificus* is between pm6 and pm7, a more posterior attachment point than in *C. elegans*. In addition, at this subcuticular ending the neurite in *P. pacificus* forms a branch that projects to the posterior end of the terminal bulb. Here it wraps around the pharyngeal lumen where the pharynx and pharyngeal-intestinal valve meet. At the same location, the distal tips of NSM also wrap around the pharynx. The location expands as food is transported into the intestine, raising the possibility that I6 and/or NSM may have a sensory function at this location that is evolutionarily derived in *P. pacificus*.

General observations on connectivity

Maps of synaptic connectivity in the pharynx of *P. pacificus* resulted in the first comparison between species of complete anatomically determined maps of wiring for a large functional unit of any nervous system (Bumbarger *et al.*, 2013). The *P. pacificus* data were compared to the first study of its kind from *C. elegans* (Alberton & Thomson, 1976). A graph theoretical treatment of these data in the nematode comparative connectomics study (Bumbarger *et al.*, 2013) was restricted to analysis of cell classes rather than individual cells, as the available *C. elegans* data neglected to identify individual neurons as postsynaptic partners in their diagrams, instead only listing the cell class. Furthermore, an ongoing re-analysis of the original transmission electron micrographs for *C. elegans* (Cook *et al.*, 2014; www.wormwiring.org) identified serious flaws that make it regrettably difficult to put much weight on comparisons with *C. elegans* at present. Specifically, they identify numerous connections missed in the original analysis, particularly those to muscle cells. This re-analysis is likely to show that *C. elegans* exhibits a higher level of conservation in connectivity with respect to *P. pacificus* and will certainly impact our view of how these circuits function.

In light of this forthcoming re-analysis, it is best to focus on patterns that are based on conservation rather than divergence in the connectivity matrix between *P. pacificus* and *C. elegans*. Several patterns can be observed where the synapse classes present are very similar, but the locations of synapses are likely to be indicative of differences in developmental signalling pathways and circuit function. For example, in *P. pacificus* the muscle cell pm3 receives synapses along its entire length, whilst in *C. elegans* the homologous muscle cell receives synapses only close to the mouth opening (Cook *et al.*, 2014; www.wormwiring.org). Similarly, the neuron M5 is presynaptic to the muscle cell pm5 along its entire length in *C. elegans*, but the synapses are restricted to the anterior portion of pm5 in *P. pacificus*.

How much does synaptic connectivity between homologous neurons change over evolutionary time? Comparative physiology in systems such as the crustacean stomatogastric ganglion or swimming circuits in leaches has demonstrated that the formation or removal of connections is not necessary for major evolutionary modifications in animal behaviour (Katz & Harris-Warrick, 1999; Newcomb & Katz, 2009; Baltzley *et al.*, 2010; Sakurai *et al.*, 2011). Instead, explanations for behavioural differences between species derive primarily from changes in the physiological properties of synapses (*e.g.*, a change in neurotransmitter) rather than a change in a connectivity matrix. However, comparative circuit descriptions in the visual systems of flies, using serial transmission electron microscopy, found that connectivity between homologous neurons was surprisingly different between distantly related species. A previous study had identified the evolutionary addition of specific photoreceptor synapses with amacrine cells that the authors hypothesised were associated with increased time resolution in visual processing (Shaw & Meinertzhagen, 1986). Comparison between *P. pacificus* and *C. elegans* has suggested a great deal of evolutionary malleability in synaptic wiring (Bumbarger *et al.*, 2013) but forthcoming higher quality *C. elegans* data may change our interpretation.

Potential connectivity

Nervous system connectivity networks are sparsely connected, meaning that only a small portion of possible connections in a connectivity matrix are realised. Spatial proximity is a clear prerequisite to the for-

mation of synapses not involving volume transmission. Potential connectivity can be defined as the subset of possible connections that meet this proximity requirement and has been explored in the context of improving understanding of the potential for synaptic plasticity (Stepanyants *et al.*, 2002).

In order to examine differences in potential connectivity between *P. pacificus* and *C. elegans*, we re-examined the anatomical data for one individual from each species and represented it as adjacency matrices that indicate whether or not neuron cell classes in the pharynx nerve ring come close enough to one another potentially to form a synapse (Table 13.1). Rather than representing observed synaptic connectivity, this table represents the potential connectivity based on proximity of neurites.

The nervous system of *P. pacificus* is more highly connected in terms of both actual and potential connectivity. Thirteen cell classes enter the nerve ring in both species and there are 78 possible adjacency relationships representing the total possible synaptic connectivity. In *P.*

Table 13.1. *Potential connectivity in the pharyngeal nerve rings of* Caenorhabditis elegans *and* Pristionchus pacificus. *A potential connection is observed if two neuron classes are adjacent to each other in at least one region of the nerve ring. White cells indicate where no connection was observed. Dark grey indicates potential connections observed in both species. Cells with a P or C indicate potential connections observed only in* P. pacificus *or* C. elegans, *respectively.*

	I1	I2	I3	I4	I5	I6	M1	M2	M3	M4	MC	MI	NSM
I1	–		P			P	P						
I2		–										P	
I3			–	P	P		P			C	P	P	C
I4	P		P	–		P					P		
I5		P			–	P		P					
I6	P		P	P	P	–	P	P	P		P	P	
M1	P		P			P	–		P		P		
M2					P	P		–					
M3					P	P	P		–				
M4			C							–	P		
MC		P	P	P		P	P			P	–		
MI		P	P		P							–	P
NSM			C									P	–

pacificus, 92.3% of these potential connections are realised, whereas in *C. elegans* they are realised in only 67.0%. In *C. elegans*, no neuron class has potential connectivity with every other neuron class. In *P. pacificus*, I2, I4, I5, I6, M3, MCL and MI come in close proximity to all other neuron classes in the pharyngeal nerve ring. All potential connections in *C. elegans* are present in *P. pacificus*, with the exception of potential I3-M4 and I3-NSM connections.

It is difficult to know the functional implications of potential connectivity comparisons, but the patterns evident between the two species at the very least demonstrate that potential connectivity should be examined together with actual connectivity in future comparative work. With larger networks or with comparisons between more taxa you can, in principle, ask whether potential connectivity places a constraint on the evolutionary formation and removal of connections. The relationship between potential connectivity and synaptic plasticity could also be investigated.

Phylogenetic comparison

Modern treatments of nematode phylogeny divide nematodes into two classes, the Chromadorea and the Enoplea (De Ley & Blaxter, 2002). Most of our knowledge of nematode nervous systems comes from the order Rhabditida, which is within the Chromadorea and includes both *P. pacificus* and *C. elegans*. Neuron number and cell body position in the pharynx is highly conserved within the Rhabditida (Zhang & Baldwin, 2000; Chiang *et al.*, 2006; Ragsdale *et al.*, 2011; Bumbarger *et al.*, 2013). Studies outside of this group are unfortunately not complete enough to evaluate homology for most individual neurons. At least 18 neurons are known to be present in the pharynx of *Ascaris suum*, a non-Rhabditida vertebrate parasite that is also placed in the Chromadorea (Cowden *et al.*, 1993). Cell bodies were identified with antibody staining and it is thought likely that the remaining two neurons may also be present in *A. suum* (Antony Stretton, pers. comm.). No reliable counts of pharyngeal neuron nuclei have been published for nematodes belonging to Enoplea.

While the fundamental pharynx nerve ring structure is conserved between *P. pacificus* and *C. elegans*, this is not the case for all nematodes. *Leptonemella juliae* (Chromadorea, Desmodorida) has six, rather than

three, major nerves interacting anterior and posterior to the central nerve ring (Hoschitz *et al*., 2001) and appears to have additional ring commissures near the mouth opening. Though more comparative work is needed to examine these additional rings, they may be homologous with the anterior metacorpus commissure and the anterior terminus of the neuron classes I1, I2, I3 and M1 in *C. elegans*. Less complete studies for a nematode belonging to Enoplea, *Longidorus leptocephalus* and *Xiphinema diversicaudatum* (Robertson, 1975, 1979) found three major nerves on each side of the nerve ring, indicating that the configuration of the nerve ring in Rhabditida is likely to be the ancestral pattern.

The presence of connections between somatic and pharyngeal nervous systems close to the mouth opening is conserved in all species examined. In *C. elegans*, *P. pacificus* and *Aphelenchus avenae* there are two bilaterally symmetric neurons (RIP) from the somatic nervous system connecting with the pharynx, suggesting that this may be conserved within the Rhabditida. In *P. pacificus*, RIP connects only to the I1 pharyngeal neurons. In *A. avenae*, it connects to both I1 and I2 (Ragsdale *et al*., 2011). In *C. elegans*, it connects with I1 and variably to I2. There are, however, at least six connections between the somatic and pharyngeal nervous systems of *Leptonemella juliae* (Hoschitz *et al*., 2001) and *Longidorus leptocephalus* (Robertson, 1979). This represents another indicator that ancestral lineages of nematodes may have had more complex nervous systems than those commonly studied.

The anatomy of pharyngeal sensory structures has not been widely surveyed in nematodes. The neuron class I1, which has subcuticular endings in both *P. pacificus* and *C. elegans*, lacks a subcuticular ending in *A. avenae* (Ragsdale *et al*., 2011). *Aphelenchus avenae* has an otherwise highly derived pharyngeal anatomy, so it is not likely that it is representative of an ancestral species. The apparent loss of this subcuticular ending in *A. avenae*, as well as the loss of a subcuticular ending in the I5 neuron of *P. pacificus*, indicates that changes in the function of sensory neurons may not be uncommon in the phylum.

In *L. juliae*, three sensory structures located in the anterior pharynx have no obvious homologues to structures found in *P. pacificus* and other members of the Rhabditida. Surprisingly, each has a glial cell and associated multiple sensory neurons. Although ciliated pharyngeal nerve endings have not been observed in the Rhabditida, an undetermined number of ciliated neurons are present in *L. juliae*. The authors speculate

that the cell bodies for these sensory structures may be found outside of the pharynx, which would also represent a significant difference from more commonly studied nematodes. In several members of the Enoplea, a number of authors have identified complex sensory structures in the anterior pharynx. The term 'endolid' was used to describe sensory structures observed with light microscopy in Dorylaimida (Siddiqi, 1970). Additionally, electron microscopy studies identified complex putatively chemosensory structures in the anterior pharynx of *X. diversicaudatum* and *L. leptocephalus* (Robertson, 1975). Ciliated neurons were observed in the endolids of *X. diversicaudatum* but not in *L. leptocephalus*. The greater complexity of sensory inputs into the pharynx of the Enoplea *vs* the Chromadorea is consistent with a greater complexity of sensory input into the somatic nervous system. Members of the Enoplea can have more sensory neurons in anterior sensilla and their bodies are typically covered in sensory setae.

The presence of a bilaterally symmetrical pair of serotonergic NSM appears to be a broadly conserved feature in nematodes (Rivard *et al.*, 2010). Antibodies against serotonin work well, making comparative observations on the anatomy of this class of neurons relatively simple. Common features of NSM neurons are the presence of a ventrosublateral process travelling on the same side of the animal as the cell body, and a process that crosses the animal ventrally, wraps around the nerve ring and then extends dorsally. Both processes contain numerous serotonin-containing synapses directed outwardly into the body cavity close to the somatic nerve ring. In most species, these processes are directed posteriorly from the cell body. In *A. suum*, the processes are directed anteriorly instead (Johnson *et al.*, 1996). NSM in *Haemonchus contortus*, another vertebrate parasite more closely related to *C. elegans*, has processes extending both anteriorly and posteriorly to the cell body (Rao *et al.*, 2010). These modifications appear to be in part tied to the somatic nerve ring, being anterior to the NSM cell bodies in large nematodes. In *P. pacificus*, NSM is highly unusual in that it does not interact significantly with the pharyngeal nerve ring and lacks dorsal processes. Furthermore, its ventrosublateral processes extend to the posterior end of the pharynx where they wrap around at the junction between the pharynx and pharyngeal-intestinal valve. Taken together, this seems to indicate that the differences in the morphology in NSM correlate with function.

Individual neuron descriptions

I1

The I1 cell class in *P. pacificus* is largely conserved in anatomy with I1 in *C. elegans* (Fig. 13.5). They are a bilaterally symmetrical pair of bipolar neurons with cell bodies located anterior and medial to those of I2 in the ventrosublateral nerves. They have the most anterior neuron cell bodies in the pharynx. An anteriorly directed process extends to a point slightly posterior to where pm2 attaches to the cuticle lining the mouth opening, where it divides into two short branches. One of these extends anteriorly to the junction between pm1 and pm2, where it forms a subcuticular ending (Figs 13.4, 13.5). The other branch extends ventrosublaterally to the periphery of the pharynx, where it forms a gap junction with the somatic interneuron RIP. This pair of gap
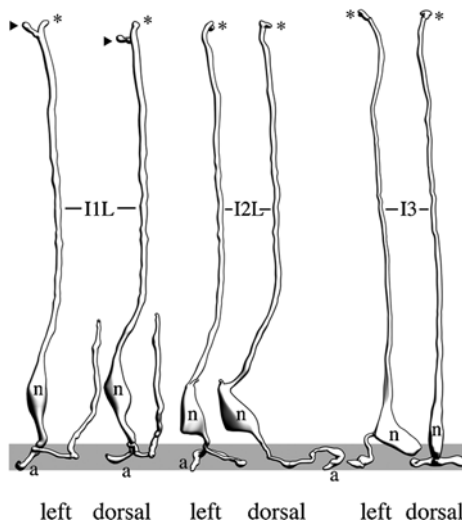

left   dorsal   left   dorsal   left   dorsal

Fig. 13.5. *3D representation of pharynx neuron cell classes I1, I2 and I3. Each neuron is represented by renderings from both the left and dorsal perspectives. The cell class name is indicated with capital letters in between the left and dorsal views. Neurite thickness is exaggerated slightly for clarity and to resemble more closely how they might appear with fluorescence microscopy. A grey bar indicates the approximate boundaries of the pharyngeal nerve ring. * = subcuticular ending. Abbreviations: a = nerve ring accessory nerve; n = nucleus; black arrowhead = gap junction with RIP neuron in the somatic nervous system.*

junctions represents the only direct connection between the pharyngeal and somatic nervous systems. For most of its length, this process of I1 is placed at the most medial position of the anterior ventrosublateral nerve. This anterior process receives input from a large number of synapses originating from M1, as well as from a smaller number of synapses originating from I2. In *C. elegans*, there are no synapses in the region of the nerve where these occur in *P. pacificus*.

The posterior process exits the cell body and enters the nerve ring. A short branch extends with variable length into the adjacent accessory nerve of the nerve ring. The main branch of the neurite projects dorsally, wrapping around the nerve ring to meet the dorsal nerve. There, it turns anteriorly and extends into pm3, terminating adjacent to the nucleus of the epithelial cell e1D. This posterior process both gives and receives synapses, with many of the outgoing synapses being directed at M3, the I1 neuron from the opposite side, and the dorsal gland cell. In *C. elegans* this process does not terminate as far anterior and synaptic output is primarily toward M2, M3 and MC.

In *P. pacificus*, presynaptic densities are not restricted to the neurites. Next to the nucleus, they form connections to the posterior regions of the pm1 muscle cells. These synapses occur where the I1 cell body is narrowing to form the anterior neurite and form a connection to a short process extending posteriorly from the ventrosublateral pm1 cell bodies. The synaptic vesicles close to the presynaptic densities are typically few in number and poorly contrasted. These motor synapses are unusual in that they are located very far from the contractile region in pm1. While no such synapses have been identified in published annotations of *C. elegans* data (Albertson & Thomson, 1976), a re-analysis has identified synapses directed towards pm1 originating at a similar location along I1 (Cook *et al.*, 2014; www.wormwiring.org).

I2

The I2 neurons (Fig. 13.5) are a bilaterally symmetrical and bipolar pair of neurons with cell bodies located in the anterior ventrosublateral nerves posterior to I1, anterior to NSM, and peripheral to MC. The neurite extending anteriorly from the cell body extends through the nerve to terminate in a subcuticular ending just anterior to the subcuticular ending of I1 and adjacent to pm1 (Figs 13.4, 13.5). This terminus differs from that of I1 in that, rather than terminating adjacent to the cuticle, it

enters and expands into the cuticle, leaving the wall of the cuticle very thin at its terminus. There are a number of tiny cellular processes extending into the tooth-like denticles lining the mouth opening. Although it is not possible with available micrographs to trace these fine processes, it is likely that they extend from I2. Along this anterior neurite, I2 receives extensive input from M1, and has a few synapses directed primarily to the pm3 and pm4 muscle cells, I1 and M1. Similar synaptic input and output in the anterior pharynx has recently been identified in the pharynx of *C. elegans* (Cook *et al.*, 2014; www.wormwiring.org).

The neurite exiting from the posterior side of the cell body enters and travels through the pharyngeal nerve ring. It crosses the midline dorsally and continues around the nerve ring to terminate at the distal tip of the accessory nerve on the side opposite from where it entered the nerve ring. This neurite receives very little synaptic input and most of the synaptic output from this neurite is directed towards the pm4 muscle.

I3

I3 is a bipolar neuron with a cell body located in the anterior dorsal nerve posterior to MI and anterior to M4 (Fig. 13.5). The anteriorly directed neurite is in contact with the dorsal gland almost its entire length, and extends to the mouth opening where it forms a subcuticular ending between the pm1 and pm2 muscle cells and at the base of the dorsal tooth. Along this process I3 receives extensive input from M1.

A short, posteriorly directed process extends from the cell body, originating just anterior to the anterior metacorpus commissure. It extends into the nerve ring, where it forms two bilaterally symmetrical branches that extend a short distance into either side of the nerve ring to terminate subdorsally. This posteriorly directed process, though short, gives and receives a number of synapses, with many of the outgoing synapses being directed towards the pm4 muscle cell.

I4

The cell body for the neuron I4 (Fig. 13.6) is located in the terminal bulb on the dorsal margin of the pharynx just to the right of the midline and at the transition between the pm5 and pm6 muscle cells. Two bilaterally symmetrical neurites exit the cell body and extend through the terminal bulb commissure to the posterior ventrosublateral nerves. Here, they travel through the isthmus to the nerve ring. As in *C. elegans*, this
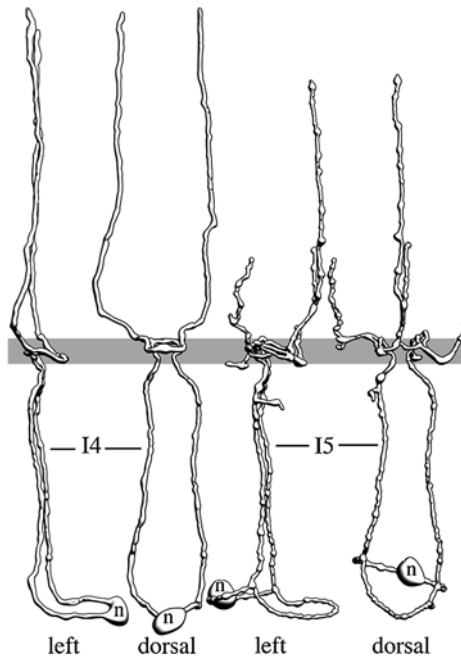
Fig. 13.6. *3D representation of pharynx neuron cell classes I4 and I5. Caption details and abbreviations as in Figure 13.5.*

region of the neurite in *P. pacificus* receives synaptic input from NSM. Upon entering the pharyngeal nerve ring, each neurite crosses ventrally and wraps around the nerve ring to the anterior ventrosublateral nerve on the opposite side. In the nerve ring, I4 does not receive synaptic input, and synaptic output is dominated by dyadic synapses to I5 and MI. These neurites continue through the corpus along the ventrosublateral nerves to terminate posterior to the buccal cavity. Synaptic input or output do not appear to be functions of the extensions through the corpus.

I5

The cell body of I5 (Fig. 13.6) is located on the ventral side of the terminal bulb close to the transition between pm5 and pm6. Two neurites originate at the cell body, one on each side. They are unusual in that they extend to the ventrosublateral nerves and continue through the terminal bulb commissure, where they fuse to form a closed loop. Where the loop passes each ventrosublateral nerve, an additional neurite branch is formed that extends through the isthmus to the nerve ring. These neurites

receive extensive input from NSM along their length and extensive input from M2 in the anterior isthmus. Upon reaching the nerve ring, they wrap around the same side to the anterior dorsal nerve where they fuse into a single process. Unlike in *C. elegans*, I5 neurites in *P. pacificus* do not fuse on the ventral side of the nerve ring. Within the nerve ring, I5 receives significant synaptic input from I4 and MI, while producing synapses that are directed primarily toward the pm4 pharynx muscle cell.

Three neurites branch from I5 in the pharyngeal nerve ring. Two processes that originate from the subdorsal sector have a highly variable morphology. In one individual where they were fully reconstructed, these neurites branched again, with one extending into the nerve ring accessory nerves and the other extending into the anterior region of pm4 on the peripheral margin of the anterior ventrosublateral nerve. In another individual, these subdorsal branches extend to the peripheral margin of the terminal bulb without migrating to the ventrosublateral side. In both individuals, the subdorsal branches direct numerous synapses towards the pm4 muscle cell. The third process originates in the anterior dorsal nerve and extends through most of the corpus, forming an additional short branch that may be a remnant of the fusion of neurites in the nerve ring. This anterior dorsal branch produces numerous synapses directed towards pm4 in the metacorpus and towards the e1D epithelial cell in the anterior region of the pharynx. It receives extensive input from I1.

Outside the pharyngeal nerve ring, neurites of I5 contain numerous varicosities having the appearance of pearls on a string. In other neurons, similar varicosities are typically associated with a synaptic density, but in I5 they occur in regions of the neuron where no synapses occur.

I6

The cell body of I6 (Fig. 13.7) is located on the left subdorsal side of the terminal bulb, slightly posterior and dorsal to that of the left M2 neuron and at the transition between the pm5 and pm6 pharynx muscle cells. Two neurites originate at the cell body.

The neurite originating on the posterior side of the I6 cell body projects to the posterior margin of the dorsal pm6 pharynx muscle cell and forms a subcuticular ending on the right subdorsal sector of the cuticle lining the pharyngeal lumen. This putative sensory ending forms junctions with the muscle cell pm6 and the marginal epithelial
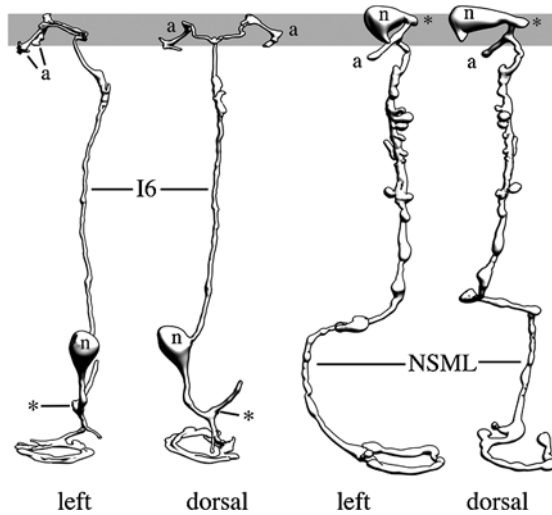
Fig. 13.7. *3D representation of pharynx neuron cell classes I6 and NSML.
* = subcuticular ending. Caption details and abbreviation as in Figure 13.5.*

cell mc3DR. In *C. elegans*, the position of this junction differs slightly, instead being located between the pm5 and pm6 muscle cells. Close to the junction, the neurite divides to form anterior and posterior branches. The anterior branch projects to the cell body of M1 where it forms a putative gap junction. This branch and connection to M1 was erroneously omitted from the drawings in the supplemental material of Bumbarger and co-workers (Bumbarger *et al.*, 2013). The branch directed posteriorly from the subcuticular ending projects to the cell body for M5, where it divides into two or more branches with variable morphology that wrap around the posterior pharynx next to the junction with the pharyngeal-intestinal valve. As the neurites wrap around the pharynx, they receive synaptic input from NSM. In one individual, synapses directed at pm8 and a single synapse directed outside of the pharynx were observed.

The neurite originating on the anterior side of the I6 cell body projects through the isthmus in the dorsal nerve with M5 and adjacent to the dorsal gland cell. In most of the isthmus, this dorsal nerve is located midway between the periphery and centre of the pharynx. Close to the somatic nerve ring, both neurons in the dorsal nerve move to a peripheral position. As it nears the metacorpus, the I6 neuron moves again to a more central position, where it projects into the pharyngeal nerve ring on the

dorsal side. Here it divides into two symmetrical branches that travel to the ventrosublateral sector of the pharyngeal nerve ring on either side. They then turn posteriorly into the accessory nerves, where they project to the distal end and terminate. These branches within the nerve ring are absent in *C. elegans*. This anterior neurite of I6 receives only a small amount of synaptic input and apparently has a primarily axonal function. It forms multiple synapses directed towards pm5 in the anterior isthmus, towards I1 in the pharyngeal nerve ring, and towards M3 in the accessory nerves.

M1

The cell body of M1 (Fig. 13.8) is located on the left subdorsal side of the terminal bulb, slightly posterior and dorsal to that of the left M2 neuron and at the transition between the pm5 and pm6 pharynx muscle cells. It occupies the equivalent position of I6 on the other side of the body.

A single neurite projects from the anterior side of the cell body and travels through the isthmus on the right subdorsal side of the pharynx, in between the pharynx muscle cell pm5 and the marginal epithelial cell mc2. Like the other dorsal neurons, as it nears the somatic nerve ring it moves to the periphery of the pharynx and moves back to a more central location as it approaches the metacorpus. M1 enters the pharyngeal nerve ring on the midway between the centre and right subdorsal region of the nerve ring. When it reaches the anterior side of the nerve ring, it splits into two branches. A short branch extends to the subdorsal corner of the nerve ring, while a longer branch projects anteriorly into the dorsal nerve. It passes medially to the anterior metacorpus commissure and on the anterior side an additional short branch forms and projects posteriorly on the peripheral side of the commissure to terminate near the pharyngeal nerve ring. This short, posteriorly-projecting branch is not found in *C. elegans*. The longer branch continues through the corpus to the transition between pm1 and pm2, where it divides into two symmetrical branches. These branches wrap around the pharyngeal lumen along either side of the animal between pm1 and pm2 until they reach the ventrosublateral nerves, where they turn to project posteriorly. In *C. elegans*, these terminal neurites of M1 project only a short distance, but in *P. pacificus* they project through the corpus to terminate at variable locations within the anterior metacorpus.
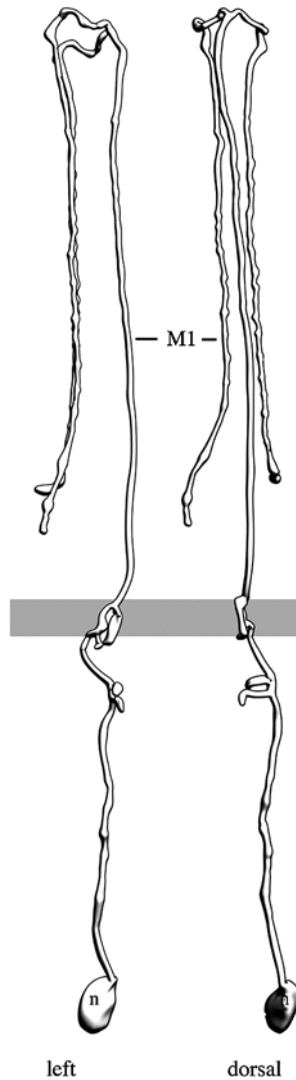
Fig. 13.8. *3D representation of pharynx neuron cell class M1. Caption details and abbreviation as in Figure 13.5.*

In both *C. elegans* (Cook *et al.*, 2014; www.wormwiring.org) and *P. pacificus*, M1 receives synaptic input from I1, I2 and MI in the pharyngeal nerve ring, and most of the output is directed towards pm1, pm2, pm3, I1, I2, I3 and e3d in the corpus. The motor output in the corpus of *C. elegans* is restricted to the anterior region of the pharynx

close to the buccal cavity, whereas in *P. pacificus* M1 forms synapses with pm3 along its entire length.

M2

M2 (Fig. 13.9) is a bilaterally symmetrical class of neurons with cell bodies lateral and slightly dorsal. They are just anterior to and ventral to those of I6 on the right side and M1 on the left side. A single neurite projects from the anterior side of the cell body into the ventrosublateral nerve on the same side of the body. From there it projects through the entire isthmus. In the anterior isthmus, the neurite exhibits a complex morphology with the appearance of a mesh network that extends between the ventral midline and the subdorsal region of the pharynx periphery. There, it makes numerous synapses directed towards the dorsal mc2 marginal epithelial cells and the pm5 pharynx muscle cell. Within the isthmus, it receives a small amount of synapses from
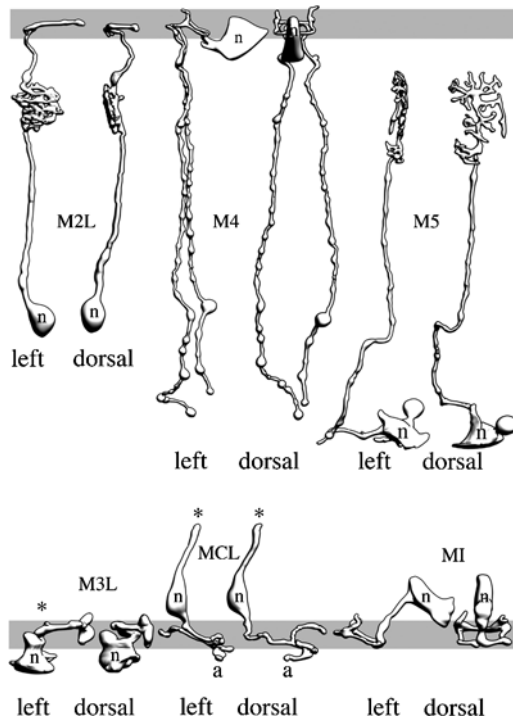

Fig. 13.9. *3D representation of pharynx neuron cell classes M2L, M3, M4, M5, MC forward and MI. Caption details and abbreviation as in Figure 13.5.*

NSM at variable locations along the neurite. As it nears the pharyngeal nerve ring, M2 resumes a more typical neurite morphology. It projects anteriorly through the medial side of the pharyngeal nerve ring. In front of the nerve ring, M2 contains between 30 and 50 microtubules that stain unusually dark in our preparations as compared to those in other neurons, giving them a distinct appearance. Upon reaching the anterior metacorpus commissure, each M2 projects along the same side of the body to the dorsal side, where it forms a junction with its partner from the opposite side. It has very little connectivity in the nerve ring, with a small number of synapses directed towards the pm4 pharynx muscle cell. In the two individuals observed, only one of the M2L received input from I1L, but M2R did not receive input.

Although the anatomy of M2 in *P. pacificus* as it projects through the anterior isthmus is quite divergent from that of M2 in *C. elegans*, the pattern of synaptic connectivity along the neuron is highly conserved, including the synapses with I1, pm4 and pm5. The single synapses received from I1 are found in the same location in both species. However, the synapses to pm5 in *C. elegans* are along the entire length of the isthmus, rather than being restricted to the anterior isthmus as in *P. pacificus*. This unusually high degree of conservation in connectivity may indicate some element of conserved function. Although the function of M2 is not well understood in either species, it has been shown to be important for isthmus peristalsis in *Panagrolaimus* (Chiang *et al*., 2006).

M3

M3 (Fig. 13.9) is a bilaterally symmetrical class of neurons with the most posterior cell bodies in the ventrosublateral metacorpus. A single neurite projects from the medial side of the cell body and passes through the inner side of the pharyngeal nerve ring. Here it forms a subcuticular ending with the cuticle lining the subventral pharyngeal gland, forming junctions with the pharynx muscles pm4 and pm5. From there it projects into the anterior metacorpus commissure and travels along the same side of the body to the dorsal nerve, meeting the M3 neuron from the opposite side. The neurite then orientates posteriorly and projects through the dorsal nerve back to the pharyngeal nerve ring, where it terminates. M3R projects through the left side of the dorsal nerve, and M3L on the right.

Close to the cell body, M3 typically receives extensive synaptic input from I1 and a smaller amount from I6. Most of these synapses originate

in the pharynx nerve ring accessory nerves that wrap around the dorsal side of the M3 cell bodies. In one individual, the synapses directed towards M3R from I1 are absent, apparently replaced by a similar number of synapses from I2. In the ventrosublateral and dorsal nerves, M3 receives additional input from I1. Along the neurite that is distal to the subcuticular ending, M3 forms multiple synapses directed towards the pm4 pharynx muscle cells. In the dorsal nerve, a small number of synapses are directed towards the dorsal gland. In both individuals of *P. pacificus* where connectivity data are available, I4 forms a small number of synapses directed towards M3R but not towards M3L. In *C. elegans*, both M3 neurons receive substantial input from I4.

In *C. elegans*, there is an additional neurite originating on the posterior side of the cell body that projects a short distance into the anterior isthmus and receives synaptic input from I4 and NSM. Both the neurite and the synaptic connections are absent in *P. pacificus*. Although there are some differences, synaptic wiring associated with M3 is generally conserved between *C. elegans* and *P. pacificus*.

M4

The cell body of M4 (Fig. 13.9) is located on the dorsal side of the metacorpus. The front of the cell body is adjacent to the pharyngeal nerve ring and it extends to the posterior end of the pm4 pharynx muscle cell. A single neurite originates from the cell body posterior to the pharyngeal nerve ring. It projects to the most medial position of the dorsal nerve and travels within it a short distance to enter the pharyngeal nerve ring. There, it divides into two symmetrical branches that occupy a medial position as they travel through the nerve ring. Each branch projects through the pharyngeal nerve ring to the ventrosublateral nerve, where it forms a short branch that extends to terminate close to the anterior metacorpus commissure. The larger branch continues projecting through the pharyngeal nerve ring, crossing the ventral midline and extending to the ventrosublateral nerve on the opposite side. Here it projects posteriorly into the isthmus. In the anterior isthmus, it briefly leaves the ventrosublateral nerve to wrap around the ventrosublateral pharynx gland cells before returning to the nerve and continuing to travel through the remainder of the isthmus. Unlike *C. elegans*, the neurites from M4 in *P. pacificus* do not enter the terminal bulb commissure. Instead, each branch continues to project posteriorly. The two branches

terminate asymmetrically, with the neurite that projects through the right side of the isthmus terminating next to the pm7 cell body. The neurite that projects through the left side of the isthmus extends posteriorly to pm7 where it crosses the ventral midline next to the neurite from M5 and travels to a position on the right side close to the posterior margin of the pharynx, where it terminates.

M4 in *P. pacificus* receives very few synapses, and the presynaptic partners vary between individuals. The only synaptic input found in both individuals was a single synapse from I4 (Table 13.1). Like *C. elegans*, M4 produces numerous synapses along the length of the isthmus directed towards the pm5 muscle cell. *P. pacificus* additionally forms a few synapses directed towards cells in the terminal bulb, including pm6 and the mc3 marginal epithelial cells.

M5

The cell body of M5 (Fig. 13.9) is located peripherally in the right subdorsal sector of the terminal bulb at the level of the most posterior pharynx muscle cell pm8. Two neurites originate from the cell body. Unlike in *C. elegans*, these neurites are not bilaterally symmetrical. A very short neurite projects from the posterior cell body. In one of the two individuals of *P. pacificus* fully reconstructed this short process receives a single synapse from NSM.

From the centre of the cell body, a single and much larger neurite projects ventrally along the right side of the pharynx lumen, meeting the terminus of M4 and travelling with it to cross the ventral midline. From here, it continues to rotate around the pharynx lumen close to M4 as it moves anteriorly to the point where the left ventrosublateral nerve and terminal bulb commissure meet. Rather than entering the commissure and remaining within the terminal bulb as in *C. elegans*, M5 in *P. pacificus* projects into the dorsal nerve alongside I6 and extends through the isthmus. Upon reaching the anterior isthmus, many processes emerge to form a network-like structure in the dorsal isthmus similar to the ones described above for M2. Within this structure, M5 produces a large number of synapses directed towards the dorsal pm5 muscle cell.

MC

MC (Fig. 13.9) is a bilaterally symmetrical class of neurons with cell bodies medial to those of I2 and posterior to I1. An anteriorly projecting

neurite exits the cell body to form a subcuticular ending at the transition between the metacorpus and corpus, forming junctions with the pm3 and pm4 pharynx muscle cells. There is no synaptic input or output along this process.

A second neurite projects posteriorly through the medial side of the adjacent ventrosublateral nerve to the pharyngeal nerve ring. It travels on the anterior side of the nerve ring to the dorsal nerve. After crossing over the dorsal midline and passing the MC neuron from the opposite side, it moves to the posterior nerve ring and continues to project to the subdorsal sector. Here, a branch forms that exits on the posterior side of the pharyngeal nerve ring. This branch has a variable morphology, typically branching one more time and forming numerous synapses directed towards the pharynx muscle cell pm4 and the adjacent mc2 marginal epithelial cell. The branch remaining in the pharyngeal nerve ring continues projecting to the ventrosublateral nerve on the opposite side of the animal from the cell body. Here it projects posteriorly, either in the accessory nerves or on the ventral side of the animal. This neurite also forms multiple synapses directed towards the muscle cell pm4 and the marginal epithelial cell mc2.

In *C. elegans*, MC neurons are cholinergic and serve to regulate the rate of pumping in the pharynx. It presumably does so through its synapses onto the mc2 marginal epithelial cells. In *P. pacificus*, MC neurons synapse directly onto the pm4 pharynx muscle cells. As the pumping rate of the pharynx in *P. pacificus* must be regulated during predatory *vs* bacterial feeding, the conservation in synaptic output makes MC the most likely candidate for performing this function. In *C. elegans*, the most important input from chemical synapses is derived from I1 neurons, whereas in *P. pacificus* it is more variable but consistently contains input from I2 neurons and not from I1. These differences in input may help to explain some of the behavioural differences.

MI

The cell body of MI (Fig. 13.9) is located within the metacorpus anterior to the cell body for M4, peripheral to that of M3 and posterior to that of the e3D epithelial cell. A single neurite projects from the medial side of the cell body and enters the dorsal nerve anterior to the anterior metacorpus commissure. It projects posteriorly past the commissure on the medial side and into the pharyngeal nerve ring. From here it projects

ventrally down the right side of the pharyngeal nerve ring, crosses ventrally, and continues around the pharyngeal nerve ring to terminate in the left subdorsal sector. In either ventrosublateral nerve, MI forms a large varicosity that tapers into a very short, anteriorly projecting, process.

In *C. elegans*, the connectivity for MI has been described as highly variable. It appears to be less so in *P. pacificus*. In both species, motor output is to the pm4 pharynx muscle cell and in both species MI is also presynaptic to M1 and I5. Chemical synaptic input was highly variable in *C. elegans* but consistently originated from I1, I3 and I4 in *P. pacificus*.

NSM

NSM (Fig. 13.7) is a bilaterally symmetrical class of neurons with cell bodies in the metacorpus posterior to those of I2 neurons and at the level of the anterior metacorpus commissure. A single process originates from the medial side of the cell body and projects to the cuticle lining the pharyngeal lumen. Here it forms a subcuticular ending anterior to where the subventral gland ducts open into the lumen. The neurite turns to project posteriorly through the medial side of the pharyngeal nerve ring without entering or forming synapses. In all other species where it has been observed, including *C. elegans*, NSM forms a branch that projects through the pharyngeal nerve ring to the dorsal side, and then into the isthmus. This makes the anatomy of NSM in *P. pacificus* highly unusual. Once the neurite reaches the cell body of M3, a short branch projects to the posterior tip of the adjacent pharynx nerve ring accessory nerve. The larger branch continues to project through the ventrosublateral nerves in the isthmus. In the anterior isthmus, NSM forms multiple synaptic densities. As in *C. elegans*, most of these synapses are directed towards the body cavity close to the somatic nerve ring. In *C. elegans*, NSM terminates within the isthmus close to the terminal bulb. In *P. pacificus*, however, NSM continues to project into the terminal bulb. They exit the ventrosublateral nerves and project posteriorly along the periphery of the pharynx until they reach the pharyngeal-intestinal valve. Here, they form several irregular branches that wrap around the pharynx with the neuron I6. Within these branches, NSM forms additional synapses directed towards the body cavity, as well as to the pm8 muscle cell. NSM appears to get feedback from I6, forming synapses directed towards I6 in the terminal bulb and receiving input from I6 in the metacorpus.

Conclusions

Microscopy and computing technologies are rapidly reducing the barrier to large-scale and higher-throughput descriptions of neuroanatomy, including maps of synaptic connectivity. Although this comparison of the pharyngeal nervous systems of the nematodes *P. pacificus* and *C. elegans* represents the first of its kind, the near future promises to transform electron microscopy image data into a bioinformatics resource.

Nematodes offer several advantages for comparative and system level studies of synaptic wiring (Jarrel *et al*., 2012; Bumbarger *et al*., 2013). Their small size will allow for both a completeness of system description and sample sizes sufficient for a comparative and experimental approach to connectomics. All neurons are identified as neurons with highly specific identities and function, and homologous neurons can be identified for most neurons between individuals of even distantly related species. A number of tools are applicable across species to provide the necessary context to bridge our understanding of network structure and function, including the ability to record simultaneously the activity of large numbers of neurons in response to controlled stimuli (Schrödel *et al*., 2013).

Therefore, continued studies of nematode connectomics and comparative neuroanatomy focused on completeness, sample size, behavioural context and an emphasis on data quality over data quantity will likely yield generalisable insight into structure-function relationships in nervous systems and play an important role in a modern approach to systems neuroscience.

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