Genetics of Behavior in *C. elegans*

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Abstract and Keywords

The nematode Caenorhabditis elegans is among the most intensely studied animals in modern experimental biology. In particular, because of its amenability to classical and molecular genetics, its simple and compact nervous system, and its transparency to optogenetic recording and manipulation, C. elegans has been widely used to investigate how individual gene products act in the context of neuronal circuits to generate behavior. C. elegans is the first and at present the only animal whose neuronal connectome has been characterized at the level of individual neurons and synapses, and the wiring of this connectome shows surprising parallels with the micro- and macro-level structures of larger brains. This chapter reviews our current molecular- and circuit-level understanding of behavior in C. elegans. In particular, we discuss mechanisms underlying the processing of sensory information, the generation of specific motor outputs, and the control of behavioral states.

Keywords: Caenorhabditis elegans, neuronal connectome, neuronal circuits, sensory information, motor outputs, behavioral states

C. elegans has a number of unique advantages for studies of neural circuits and behavior. It is unusually amenable to genetic analysis, being easily grown on microbiological growth media, and having a short generation time of only 3 days. Moreover, individual *C. elegans* exist as either self-fertile hermaphrodites or males; thus, mutant strains can be isolated and propagated through self-fertilization, while cross-fertilization allows mapping and epistasis analysis. Behavioral assays are robust, and they have been used to identify many genes affecting nervous system function and development. *C. elegans* also has a compact and well-annotated genome (the first animal genome to be sequenced [*C. elegans* Sequencing Consortium, 1998]), and generation of transgenic animals is rapid and efficient. *C. elegans* research has played a pioneering role in the development of important techniques, including RNA interference (Fire et al., 1998) and the use of

fluorescent protein reporters (Chalfie et al., 1994), which have since found a wide range of applications in many other organisms.

In this chapter, we have discussed some specific contributions to the field of neurobiology made using the *C. elegans* model. It should be noted that here we primarily discuss discoveries made using the nervous system of hermaphrodite animals. Studies using sex-specific neurons in male *C. elegans* have revealed interesting insights into, for example, how the complex sequence of steps in male mating behavior coordinates particular sensory inputs with distinct motor outputs, and is discussed in detail elsewhere (Liu & Sternberg, 1995; Portman, 2007, 2017; Garcia & Portman, 2016).

Optogenetic Approaches

Because of its transparent body, its compact nervous system, and ease of transgenesis, *C. elegans* is particularly amenable to optogenetic recording and manipulation of neural activity. Indeed, *C. elegans* was the first animal in which an optogenetic indicator, the genetically encoded calcium indicator (GECI) cameleon (Miyawaki et al., 1997), was used to record neural activity (Kerr et al., 2000). GECIs have subsequently become widely used to monitor the activities of many identified neurons and muscle cells in both immobilized and freely moving preparations (Faumont & Lockery, 2006; Ben Arous et al., 2010). *C. elegans* also has played a critical role in the development of optogenetic effectors; while the first optogenetic effectors were developed and validated in *Drosophila* (Zemelman et al., 2002), channelrhodopsins were first used to control the behavior of an animal in *C. elegans* (Nagel et al., 2005). Systems for automated control of patterned illumination have made it possible to carry out precise optical control of neural activity in freely behaving nematodes (Leifer et al., 2011; Stirman et al., 2011). Optogenetic methods for analyzing synaptic connectivity, in particular GFP reconstitution across synaptic partners (GRASP) (Feinberg et al., 2008), have also been developed in *C. elegans* and are now widely applied in larger nervous systems.

The *C. elegans* Connectome

C. elegans is the first, and currently still the only, animal with a largely complete neuronal connectome. Like many nematodes, *C. elegans* has a compact nervous system with a small and invariant number of neurons. As a consequence, work began in the mid-1970s to characterize the *C. elegans* nervous system at the level of individual cells and synapses. Similar to today's fly and mouse connectome projects, connectivity was determined through reconstruction of serial electron micrographs; however, due to the lack of computing power, sectioning, image registration, and process tracing were carried out manually by technicians of remarkable proficiency, Nichol Thomson and Eileen Southgate (Emmons, 2015). Using this approach, the pharyngeal nervous system (composed of the 20 neurons which lie completely within the pharynx) was first reconstructed (Albertson & Thomson, 1976), followed by the entire nervous system 10 years later (White et al., 1986), with gaps in the ventral nerve cord filled in 2006 (Chen et al., 2006). Together, this work generated an essentially complete wiring diagram for the adult hermaphrodite, with 302 identified neurons connected by 7,800 chemical synapses and 900 gap junctions. Recently, the connectivity of the adult male *C. elegans*, with 383 neurons, was reconstructed using a similar approach (Jarrell et al., 2012).

In addition to these connectivity maps, many other aspects of the *C. elegans* nervous system have been characterized at the level of individual neurons. For example, the invariant embryonic and postembryonic lineages of all somatic cells, including neurons, have been completely determined (Sulston & Horvitz, 1977; Sulston et al., 1983). In addition, the patterns of neurotransmitter usage have been largely described for all neurons; in particular, the releasing neurons for the classical neurotransmitters acetylcholine, glutamate, and GABA have been comprehensively mapped (McIntire et al., 1993b; Serrano-Saiz et al., 2013; Pereira et al., 2015). Likewise, cells releasing one of four monoamine neuromodulators—dopamine, serotonin, tyramine, or octopamine—have been identified, as have most of the neurons expressing monoamine receptors (Duerr et al., 1999; Hobert, 2013). The extensive neuropeptide systems of *C. elegans*, consisting of hundreds of distinct receptors and their ligands, are less completely characterized, though efforts to map these pathways are ongoing (Kim & Li, 2004; Husson et al., 2007). Both monoamines and neuropeptides are thought to act largely extrasynaptically, and the topologies of these neuromodulatory connectomes appear to be distinct from those of the wired synaptic and gap junction networks (Bentley et al., 2016). Finally, through the use of genetically encoded probes, whole-brain imaging studies have begun to map the functional connectivity of the *C. elegans* nervous system and to relate this information to the structural connectome as well as to behavior (Kato et al., 2015; Nguyen et al., 2016).

Although the *C. elegans* nervous system is orders of magnitude smaller than those of even relatively simple models such as *Drosophila*, analysis of the worm connectome structure has yielded useful insight into the structures of larger brains. For example, motif analysis has identified overrepresented wiring patterns in the worm connectome likely to

represent microcircuits with conserved computational functions (Milo et al., 2002). In particular, variations of the feedforward circuit have been shown to be overrepresented not only in the worm connectome (Reigl et al., 2004; Azulay et al., 2016) but also in more complex neural systems such as the human cerebral cortex (Varshney et al., 2011). Organizational principles of the worm nervous system also appear to be conserved in more complex neural networks. For example, the *C. elegans* nervous system was the first biological example of a small-world network, exhibiting the properties of higher-than-expected clustering and shorter-than-expected path length (Watts & Strogatz, 1998). Subsequently, a number of much larger nervous systems have been shown to share the scale-free characteristic of small-worldness (Bassett & Bullmore, 2006; Muldoon et al., 2016). Similarly, both the *C. elegans* connectome and the human cerebral cortex share the property of rich club organization, in which the highest degree (most highly connected) hubs are more highly interconnected to one another than lower degree nodes (Bullmore & Sporns, 2012; Towlson et al., 2013). However, in *C. elegans* the nodes of the rich club are individual neurons, whereas the nodes in the human brain are cortical areas with orders of magnitude more neurons than the entire worm connectome. Together, these findings demonstrate that the *C. elegans* connectome can serve as a prototype for understanding both micro- and macro-scale properties of bigger nervous systems (Fig. 1).

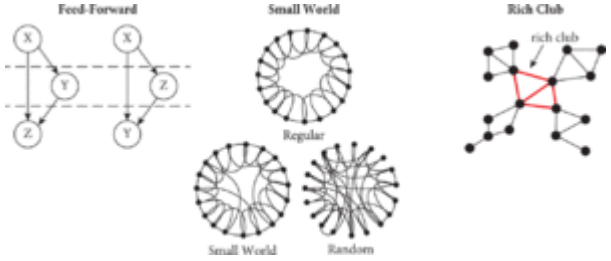

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Figure 1 Examples of a feedforward loop motif, small-world network, and rich club organization. For the feedforward loop depicted here, X, Y, and Z are neurons in different layers of the connectome, for example, sensory, inter/premotor, and motor neurons. (Figure adapted from Azulay et al., 2016.) Small-world networks feature locally connected, closely tied modules, with shortcuts between modules to allow efficient communication. (Figure depicting an example of a small-world network is adapted from Watts and Strogatz, 1998.) Lastly, rich club organization represents the interconnection of highly connected hubs.

(Figure showing an example of rich club organization is adapted from Sporns, 2013.)

Sensory Inputs

C. elegans uses a diverse range of mechanical (touch, pain, proprioception), gustatory (nonvolatile), and olfactory (volatile) cues to interpret its surrounding with respect to food availability and danger, and to respond appropriately by changes in a variety of motor outputs and other changes, such as entry to or exit from dauer, a type of stasis that allows survival of harsh conditions. A major output is, of course, the body wall muscles, required for locomotion (attraction, avoidance, food slowing), but sensory input also generates changes in the activity of other muscular structures, required for feeding, defecation, egg laying, and mating (Fig. 2).

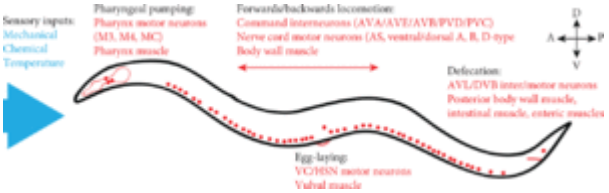

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Figure 2 C. elegans detects a wide range of sensory inputs and responds by changing a variety of motor outputs. Some examples are detailed here, along with major neurons and muscle groups required to modulate outputs.

Mechanosensation: Neurons and Circuits

Most animals can identify at least two distinct types of mechanical stimulation, gentle touch (innocuous; pleasant) and harsh touch (painful; unpleasant), and *C. elegans* is no exception. Gentle or harsh touch to the head or body results in an escape response, in the form of movement in the opposite direction or a more subtle steering response to alter the direction of locomotion. At least 65 mechanosensory neurons have thus far been identified (see Schafer, 2015, for a comprehensive list), and the majority of these are implicated in these readily assayed avoidance behaviors. Distinct groups of neurons detect distinct types of stimulus, and in distinct parts of the body (Goodman, 2006; Bounoutas & Chalfie, 2007; Schafer, 2015).

The touch receptor neurons (TRNs, Fig. 3) respond to gentle body touch, with three anterior (ALML, ALMR, and AVM) and three posterior (PLML, PLMR, and PVM) neurons accounting for touch sensation in the corresponding halves of the body. Since the demonstration of their role in gentle touch, by laser ablation, and the isolation of the first Mec (mechansensation defective) mutants (Chalfie & Sulston, 1981; Chalfie et al., 1985), the TRNs have become an important and well-characterized model for understanding not only mechanosensation but also learning, aging, and neurodegenerative disease (Giles & Rankin, 2009; Peng et al., 2011; Alexander et al., 2014).

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Figure 3 C. elegans has six gentle (low-threshold) touch receptor neurons.

Harsh body touch is detected by a different set of neurons (Fig. 4). BDU and SDQR act anteriorly, with smaller roles played by AQR and ADE; PVD and PDE act posteriorly, FLP acts in the head, and PHA and PHB act in the tail (Way & Chalfie, 1989; Li et al., 2011; Zou et al., 2017); the TRNs also play a role in detection of harsh touch (Suzuki et al., 2003; Chatzigeorgiou et al., 2010b).

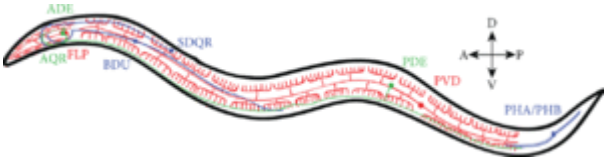

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Figure 4 C. elegans detects harsh (high-threshold) touch via several neurons located throughout the body.

Nose touch sensation likewise requires a distinct set of neurons, with ASH and FLP playing major roles, and OLQ and CEP making smaller contributions (Kaplan & Horvitz, 1993; Chatzigeorgiou & Schafer, 2011) (Fig. 5). While the TRNs appear to be nearly identical functionally, the neurons implicated here include several distinct classes. The ASH neurons are bilaterally symmetrical polymodal nociceptors, also detecting aversive chemical and osmotic stimuli (Kaplan & Horvitz, 1993), with single ciliated endings in the amphid sensilla. The FLPs are multidendritic, with extensive arborization and unexposed ciliated endings. They are likewise polymodal nociceptors, also responding to noxious heat (Liu et al., 2012). OLQ and CEP, of which there are four each, are in the outer labial sensilla.

Each of these sensory circuit modules feeds into a common downstream target, the command interneuron circuit that controls locomotion, but in a subtly different way, resulting in distinct motor outputs. The importance of this was underlined by an elegant study (Li et al., 2011). By counting body swings executed during an escape response, they showed that gentle touch and harsh touch elicit quantitatively distinct behavioral outputs, with harsh touch responses being more extensive. Interestingly, they found that whereas the command interneuron PVC is stimulated by both gentle and harsh touch, the amplitude (and thus duration) of calcium responses was much higher for harsh touch. This increased activity of PVC would serve to tip the balance of the command circuit in favor of reversal, thereby increasing the duration. Animals in which the harsh touch neurons were ablated (while the TRNs were left intact), on the other hand, showed no evidence of distinguishing between harsh and gentle stimulation, with both the behavioral response and PVC calcium response resembling the response to gentle touch.

Summation of information, by convergence of multiple sensory neurons onto a common downstream target, is a common theme in sensory circuits in a wide range of systems, and it serves to improve signal reliability and eliminate noise. The involvement of a group of neurons in each sensory modality suggests that the same is true here. Convergence of outputs from bi-, tri-, or quadrilaterally symmetrical groups, meanwhile, adds spatial

information, such that coincidence detection can potentially be used to distinguish between, for example, the squeezing effects of entering a space that is too small versus brushing against an object on one side, which would require distinct locomotion outputs. However, the sensory circuitry, and in particular, the involvement of gap junctions, suggest a much greater degree of processing complexity. This is illustrated by the nose touch neurons. The FLP, OLQ, and CEP neurons are all coupled by gap junctions to a "hub" neuron, RIH (Fig. 5). FLP responds to harsh touch, and it outputs directly to the command circuit. OLQ and CEP lack this direct connection, but their stimulation by gentle touch facilitates gentle touch response in FLP. In the absence of stimulation, they act as sinks to neuronal activity in FLP, via gap junction shunting, inhibiting its response to gentle nose touch (Chatzigeorgiou & Schafer, 2011; Rabinowitch et al., 2014). The circuit thus acts as a coincidence detector and thereby uses spatiotemporal information, in combination with stimulus strength, to distinguish multiple modes of sensory stimulation warranting an escape response from those which do not. These auxiliary neurons also output to other motor functions, when stimulated alone. The OLQs and IL1s function in the more subtle response of head withdrawal (Hart et al., 1995), whereas the CEPs detect textural changes on entry into a bacterial lawn and, via dopamine release, act on the locomotion circuit to bring about the slowing response to food (Sawin et al., 2000). Thus, the organization of this circuit allows multiple different types of motor response, as appropriate to the precise nature of the stimulus.

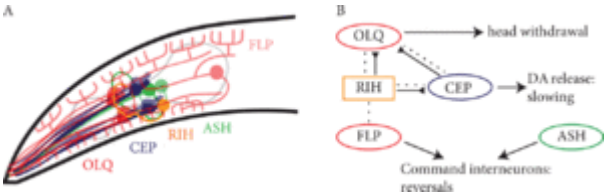

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Figure 5 The *C. elegans* nose touch circuit requires a distinct set of mechanosensory neurons. The diagram indicates neurons known to detect nose touch, particularly ASH, FLP, OLQ, and CEP, together with hub interneuron RIH. (*A*) Position of neurons in the head. (*B*) Circuit of nose touch neurons. OLQs function to mediate head withdrawal in response to nose touch, whereas the dopaminergic CEP neurons act via dopamine release onto the locomotion circuit to cause slowing on a bacterial lawn. FLP and ASH synapse directly onto command interneurons to stimulate reversals in response to nose touch. Triangle arrowheads indicate synaptic connections; dashed lines indicate gap junction connections.

A particular challenge in elucidating the mechanisms of mechanosensation has been the identification of the molecules responsible for mechanotransduction. The existence of readily assayable mechanosensory behaviors, combined with genetic tractability and the ease of cultivating large numbers, makes *C. elegans* a powerful model in which to screen for genes encoding mechanotransducers. The ability to demonstrate cell-autonomous rescue in the appropriate cell type, and to show a role in mechanically evoked responses in that cell (using genetically encoded calcium indicators or electrophysiology), provides further evidence. The existence of polymodal neurons such as ASH and PVD opens the door to demonstrating that the role of a protein

is mechanosensation specific, as opposed to generally affecting activity of the neuron, whereas heterologous expression can allow demonstration of sufficiency.

MEC-4 and MEC-10, which were identified in a screen for gentle touch–defective mutants (Chalfie & Sulston, 1981), are a classic example of the utility of a forward genetic approach. They are founding members of the DEG/ENaC family of amiloride-sensitive sodium channels (DEG for degenerin, as gain-of-function [gf] mutations in MEC-4 (MEC-4(d)) cause degeneration; Chalfie & Au, 1989). As such, they have been instrumental in identifying family members in other species. MEC-4 and MEC-10 appear to be interdependent in the gentle touch response. MEC-4 is expressed only in the TRNs and is essential for gentle touch–evoked calcium transients (Suzuki et al., 2003) and currents (O'Hagan et al., 2005) in the TRNs, and for the behavioral response. MEC-10, on the other hand, is important but not essential for behavioral and calcium responses (Chatzigeorgiou et al., 2010a) and is insufficient for mechanoreceptor currents alone (O'Hagan et al., 2005). When expressed in a heterologous system, neither is sufficient to evoke a mechanosensory response, although MEC-4(d) channels do produce constitutively active, amiloride-sensitive currents (Goodman et al., 2002). However, coexpression of both in oocytes was recently shown to be sufficient for sheer stress-evoked currents (Shi et al., 2016), suggesting that, while MEC-4 homotrimers form a functional channel, an additional subunit type is required for this channel to be mechanically gated. The fact that MEC-10 is not essential for gentle touch perhaps suggests that MEC-4 may also trimerize with another, as yet unknown, subunit, also capable of conferring mechanosensitivity.

As Table 1 shows, DEG/ENaCs have also been implicated in other mechanosensory neurons. MEC-10, for example, is also implicated in harsh touch, in FLP and PVD, along with DEGT-1, again suggesting a heterotrimeric organization. In addition, however, several diverse transient receptor potential (TRP) family members are also implicated, either directly or indirectly in mechanotransduction. TRP-4, for example, appears to play multiple mechanosensory roles in multiple neurons (Li et al., 2006; Kindt et al., 2007a; Kang et al., 2010), whereas OSM-9 plays a more general role; although it is found in several mechanosensory neurons, in ASH it is also required for response to chemical stimuli (Tobin et al., 2002) and is not required for mechanoreceptor potentials (Geffeney et al., 2011). The *C. elegans* genome also encodes a homolog of the mechanotransduction channel Piezo (Kim et al., 2012), and members of the transmembrane channel-like (TMC) family, members of which are implicated in cochlea hair cell function (Kurima et al., 2002), so these are good candidates for future investigation. Pannexins have been widely implicated in mechanosensory mechanisms, including neuropathic pain (Jeon & Youn, 2015), and the homologous innexin family has 25 members in *C. elegans*. Both UNC-7 and at least one other unidentified innexin function in mechanosensation in the TRNs (Sangaletti et al., 2014; D. S. W. & W. R. S., in preparation).

Table 1 Molecules Implicated in Mechanosensation

Family	Protein	Neurons	Sensory Stimulus	References
DEG/ENaC	MEC-4	TRNs	Gentle body touch	Chalfie & Sulston, 1981
	MEC-10	TRNs, PVD, FLP	Gentle body touch Harsh body touch	Chalfie & Sulston, 1981 Chatzigeorgiou et al., 2010a
	DEGT-1	PVD, FLP	Harsh body touch	Chatzigeorgiou et al., 2010a
	DEG-1	ASH	Nose touch	Geffeney et al., 2011
	DELM-1, DELM-2	OLQ and IL1 glial socket cells	Nose touch (indirect)	Han et al., 2013
TRP	TRP-4 (TRPN)	CEP, ADE, PDE	Nose touch, food slowing	Kang et al., 2010; Kindt et al., 2007a
		DVA	Proprioception	Li et al., 2006
	TRPA-1 (TRPA)	OLQ	Nose touch	Kindt et al., 2007b

	OSM-9 (TRPV)	ASH, FLP, OLQ	Nose touch (nonspecific)	Colbert et al., 1997
Innexins	UNC-7	TRNs, PVD	Gentle, harsh body touch	D. S. W. & W. R. S., in preparation
	Unknown	TRNs	Negative pneumatic pressure	Sangaletti et al., 2014

Chemosensation

There are at least 38 chemosensory neurons, located mostly at the sensory openings of the amphids and inner labia in the head, and the phasmids in the tail. In addition, the oxygen- and carbon dioxide–sensing neurons (AQR, PQR, URX, and BAG) have enclosed endings within the body. These are ciliated neurons, of diverse morphology; as a general rule, the endings of gustatory neurons are exposed directly to the exterior (e.g., ASH, ASI, and ASK), whereas those that are only olfactory (AWA, AWB, and AWC) are in close proximity but enclosed by sheath cells (see Bargmann, 2006; Hart & Chao, 2010, for reviews). Each expresses a subset of receptors and detects a specific set of attractants, repellants, and pheromones.

ASE plays a major role in detecting water-soluble attractants, whereas ADF, ASG, ASI, ASJ, and ASK play more minor roles, revealed following laser ablation of ASE (Bargmann & Horvitz, 1991). The ASH nociceptors function in aversion to heavy metals, bitter tastes, and high osmotic strength (Hilliard et al., 2004), as well as nose touch, as discussed earlier.

Distinct neurons are required for attraction versus repulsion. For example, benzaldehyde is sensed by both ASH and AWC; the former functions in repulsion, and the latter in attraction. Thus, the valence of a sensory cue is hardwired into the circuit. This was elegantly demonstrated by heterologous expression of the diacetyl receptor, ODR-10, which functions in attraction in AWA. When ODR-10 is expressed in AWC, diacetyl is also attractive, while if it is expressed in AWB, it becomes repulsive (Troemel et al., 1997; Wes & Bargmann, 2001).

Although the majority of chemosensory neurons appear to be symmetric pairs, expressing the same genes and exhibiting the same functions, there are two intriguing examples of asymmetry, with distinct functional outcomes. The AWCs both detect benzaldehyde, whereas butanone is only detected by one of the pair, and this asymmetry is necessary for distinguishing between these odors (Wes & Bargmann, 2001; Alqadah et al., 2016). In this case, then, asymmetry serves to increase the repertoire of odors that can be distinguished, via activation of characteristic signature combinations of sensory neurons gradients. Interestingly, unlike most bilateral neuron pairs in *C. elegans*, the left and right AWC neurons are not interconnected by gap junctions, a property shared with the ASE neurons (see later discussion). This raises the possibility that other sensory neurons whose bilateral homologs are not electrically coupled (e.g. ASG) might also show lateral asymmetry.

The ASE neurons, on the other hand, seem to be functionally opposite in their response to NaCl. ASEL is an ON-cell, stimulated by upsteps in NaCl concentration, whereas ASER is an OFF-cell, stimulated by downsteps (Suzuki et al., 2008). When negotiating a gradient, calcium responses recorded in ASER are complex, with information about the current concentration in relation to the “set point” (the previous cultivation concentration) being encoded in a combination of amplitude and frequency (Luo et al., 2014), so further

investigation is needed to fully understand the relationship between ASER and L. But the utility of such an on/off mechanism for negotiating gradients again leads us to question whether other examples exist. An alternative strategy is the integration of sensory input from the amphids in the head and the phasmids in the tail, as seen in the avoidance of the repellent SDS (Hilliard et al., 2002). ASH and ASK in the head, and PHA and PHB in the tail, detect SDS and act antagonistically on the motor circuit, effectively specifying a spatial map of the chemical environment and effecting an appropriate locomotory response. PHA and PHB detect a range of noxious stimuli (Zou et al., 2017), so this type of integration of anterior and posterior information could be a general strategy for decision making with respect to noxious cues, whereas comparison with a “set point” represents a better strategy for negotiating food cue gradients to find the optimal concentration.

Chemosensory Molecules

The worm genome encodes a vast array of chemosensory receptors, accounting for almost 10% of the genome, of which the majority are G protein–coupled receptors (GPCRs). ODR-10, the receptor for diacetyl, for example, was identified in a forward genetic screen for chemotaxis defects, and it was the first GPCR in any organism to be functionally characterized (Sengupta et al., 1996). Each chemosensory neuron expresses multiple receptors, in contrast with olfaction in flies and vertebrates, where each olfactory neuron type expresses a single receptor type. Thus, the sensory versatility of each neuron compensates for the small number of sensory neurons. Downstream of these GPCRs are a family of 21 G alpha subunits (Jansen et al., 1999), with differing downstream targets (e.g., cGMP-gated channels, TRPV channels), and thus multiple potential targets for specific adaptation and learning.

Thermosensation

Thermotaxis illustrates the power of the *C. elegans* nervous system: A worm senses the ambient temperature, associates this with presence of food, and then, when placed on a temperature gradient, accurately migrates toward and tracks the previous cultivation temperature (Mori & Ohshima, 1995). The amphid neuron AFD plays a central role in temperature sensing; if worms are cultivated at 20°C and then subjected to 2° upsteps between 15°C and 25°C, AFD begins to respond as the previous ambient temperature is approached (at 19°C), and the amplitude of responses increases incrementally as the temperature increases. AWC also responds to temperature changes, although it is less clear whether this depends on input from other neurons. Three receptor guanylate cyclases act redundantly to sense temperature in AFD, and they produce cGMP that activates cyclic nucleotide gated channels. Additional genes may also function in thermosensation, potentially as direct temperature sensors. One candidate is the GPCR SRTX-1, which is required for isothermal tracking and is expressed in AFD and AWC. AFD and AWC have both excitatory and inhibitory outputs to the interneuron AIY, and it is the balance between these that determines the precise temperature-correlated activity of AIY and thus thermophilic drive (Garrity et al., 2010; Ohta & Kuhara, 2013; Aoki & Mori, 2015). In addition, though, noxious temperature is detected by the nociceptive neurons; FLP senses heat, whereas PVD senses cold, and in both cases TRP family members (OSM-9 and OCR-2 in FLP; TRPA-1 in PVD) have been implicated (Chatzigeorgiou et al., 2010b; Chatzigeorgiou & Schafer, 2011). Interestingly, AFD also functions in detection of noxious heat, using distinct guanylate cyclases, and coupling to distinct downstream circuits, for thermotaxis and thermonociception (Liu et al., 2012).

Learning, Memory, and Decision Making

For an organism to survive in a changing environment, its nervous system must have the ability to adapt—to balance food finding and reproduction with threat tolerance, and to adjust the balance of the system in this respect, based on experience. Decoding the underlying molecular and circuit mechanisms is extremely challenging, but the relative simplicity of the *C. elegans* nervous system has allowed approaches that have been instrumental in advancing our understanding. Despite its relatively small number of neurons, *C. elegans* is capable of modifying mechanosensory, olfactory, gustatory, and thermosensory behaviors through associative and nonassociative learning, as a result of sensory experience (Hedgecock & Russell, 1975; Rankin, 1991; Colbert & Bargmann, 1995; Saeki et al., 2001).

When animals are exposed to a high concentration of an attractive cue, in the absence of food (starvation, a negative cue), associative learning results, and the attraction is gradually lost over a period of an hour or two. This plasticity is selective for that particular cue, even when several cues are detected by overlapping sensory neurons (e.g., Colbert & Bargmann, 1995). The basic requirement for such a mechanism is that

sensory cue and food context are convergently represented to allow association. An emerging theme is that association may occur via a downstream neuromodulatory interneuron, which then acts upon the sensory neuron to change either its dynamic range or its synaptic output. An example of this (Cho et al., 2016) illustrates the functional distinction between sensory adaptation to butanone (nonassociative learning) and aversive (associative) learning following conditioning with butanone and food deprivation. The former increases the dynamic range of AWC^{ON} calcium response to butanone, irrespective of pairing with reward or punishment, suggesting that information about odor history is stored in AWC itself. Pairing with food deprivation, on the other hand, requires input from the downstream interneuron AIA, and other neurons, in the form of insulin signaling, to bring about changes in gene expression and thus alter synaptic output.

A second recent study (Ghosh et al., 2016) elegantly demonstrates the role of a second interneuron, RIM, in risk-reward multisensory decision making. RIM receives inhibitory input from sensory neurons activated by attractive odors (e.g., diacetyl, from AWA) and excitatory inputs from sensory neurons activated by aversive stimuli (e.g., high osmolarity, from ASH). It in turn provides inhibitory input to forward command neurons and excitatory input to backward command neurons. Animals were surrounded by a high-osmolarity barrier, with a source of diacetyl placed outside it. Well-fed animals stay within the barrier, while starvation increases the willingness to ignore the aversive cue to reach the (food-indicating) attractive odorant. In well-fed worms, RIM secretes tyramine, which potentiates ASH activity via the TYRA-2 receptor, resulting in a positive feedback loop. Food deprivation inhibits RIM activity and thus suppresses ASH potentiation, effectively pushing the balance of the circuit in favor of attraction to diacetyl.

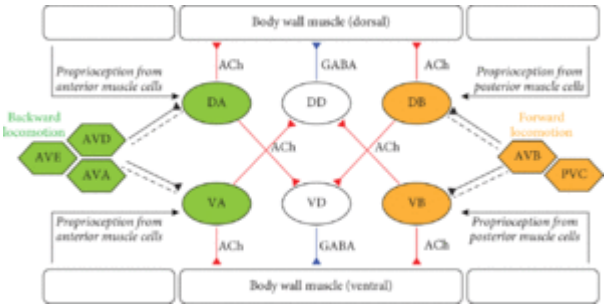
Motor Outputs

The Locomotion Circuit

C. elegans moves in a sinusoidal fashion, via dorso-ventral bends, the wavelength and frequency of which depend on the resistance (viscosity) of the surroundings. The 95 rhomboid body wall muscle cells are arranged as staggered paired rows in four quadrants (dorsal: left and right; ventral: left and right), each receiving multiple inputs from the 75 motor neurons. Corresponding muscle cells contract and relax in a reciprocal fashion; for a dorsal bend to occur, for example, the dorsal muscle cells contract while their ventral counterparts relax. For movement to occur, these waveforms must be propagated sequentially to neighboring muscle cells, along the length of the animal, in the appropriate direction; and for movement to be sustained, oscillation between these contracted and relaxed states is required. Recent advances have helped in the quest to

explain how these basic requirements of the motor circuit are achieved (for reviews, see Gjorgjieva et al., 2014; Zhen & Samuel, 2015).

The ventral nerve cord (VNC) contains most of the motor neurons that drive undulatory locomotion. These neurons consist of five classes: A, B, D, VC, and AS, of which A, B, and D are further divided into subclasses that innervate dorsal or ventral muscle cells (e.g., VA, DA). Undulating movement is largely organized by the A- and B-type cholinergic (excitatory) neurons and the D-type GABAergic (inhibitory) neurons (Fig. 6). The A- and B-type neurons are postsynaptic to five pairs of premotor interneurons, known as the command interneurons. Ablation studies have shown that AVA, AVD, and AVE are required for backward movement, and they connect mainly to the A-type motor neurons; AVB and PVC are required for forward movement and connect mainly to the B-type motor neurons. The D-type GABAergic neurons are postsynaptic to the A- and B-type neurons and innervate muscle on the opposing side of the body, resulting in contralateral inhibition, although neither the DDs nor VDs are essential for bending (Donnelly et al., 2013), so their role may be modulatory.



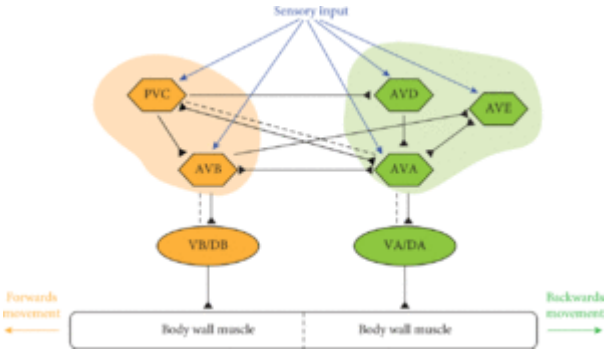
Click to view larger

Figure 6 The *C. elegans* locomotion circuit. Command interneurons AVE, AVD, and AVA drive backward locomotion by acting onto VA/DA neurons, whereas AVB and PVC drive forward locomotion by acting onto VB/DB neurons. These A- and B-type motor neurons, in turn, stimulate muscle via acetylcholine signaling, with VA/VB neurons innervating ventral muscle and DA/DB neurons innervating dorsal muscle. The D-type neurons are postsynaptic to A- and B-type neurons, and they signal to muscle on the opposing side via inhibitory GABAergic signaling to modulate bending. Proprioceptive cues from anterior or posterior muscle cells also stimulate activity of A- and B-type neurons to drive movement. Triangle arrowheads indicate synaptic connections; dashed lines indicate gap junction connections.

The cholinergic motor neurons have long neurites devoid of synapses, which, importantly, extend in opposing directions; those of A-type neurons extent anteriorly, while those of B-type neurons extend posteriorly. During forward movement, when B-type motor neurons sense the bending of more anterior regions, they drive local bending (Wen et al., 2012). Thus, a bend in a specific location causes a precisely delayed bend at a precise location posterior to this. This simple proprioceptive relationship can explain propagation of the wave in a posterior direction, providing the thrust for forward movement. Indeed, recent modeling (Kunert et al., 2017) suggests that this proprioceptive feedback, coupled with the bistability of the network, is sufficient to generate the rhythmic activity required to sustain movement, and that the long-sought central pattern generator may not exist. The opposite orientation of the A-type neurons presumably has the reverse effect, so the direction of movement is determined by whether the A- or B-neurons are exerting control (Fig. 6). The decision

between forward and backward movement is thus determined by the balance of power between these opposing forces, which in turn depends on the balance of power between the command interneurons, especially AVA and AVB.

AVA and AVB are reciprocal in their activity levels; an increase in AVA activity coincides with backward movement, whereas increased AVB activity coincides with forward movement, and vice versa. AVA and AVB control the activity levels of the A- and B-type motor neurons, respectively, via substantial UNC-7-UNC-9 gap junctions (Starich et al., 2009; Kawano et al., 2011) (Fig. 7), with the result that they follow the same activity patterns (these gap junctions also serve to shunt current, reinforcing the inactive state). The command interneurons are synaptically and electrically interconnected in a complex manner. Although there is no obvious reciprocal wiring to explain how cross inhibition between the forward and reverse command interneurons is achieved, it is clear that this bistable network presents multiple opportunities for intervention by sensory input or neuromodulation.



Click to view larger

Figure 7 Command circuit for locomotion in *C. elegans*. The command interneurons synapse directly onto A- and B-type motor neurons of the ventral nerve cord, and they are also extensively connected to each other. In this figure, only some of the synaptic connections between the command interneurons are shown. For details, see "The Mind of a Worm" (White et al., 1986), available online via WormAtlas (http://www.wormatlas.org/ver1/MoW_built0.92/toc.html).

An ambitious project to simultaneously image the activity of a large number of neurons (Kato et al., 2015) provided evidence for coordinated dynamic network activity. They showed that many neurons, across the sensory, premotor, and motor groups, were engaged in specific collective activity states, and that these signature activity profiles could be used to accurately predict the locomotory activity that the worm was engaged in. These states are recurrent and correspond to repeated actions of the worm, providing a common framework on which sensory cues act. This is important, because the state of the network at the time that the stimulus is received will determine how it responds, as Gordus et al. (2015) demonstrated for the interneuron circuit downstream of AWC. Whereas AWC unfailingly responds to an odor stimulus, the behavioral response is less reliable, and the reason for this lies in the activity state of the circuit. AIB, RIM, and the command motor neuron AVA are strongly connected by chemical and electrical synapses, and have bistable correlated activity states. Their response to the odor stimulus thus depends on their collective activity.

Locomotion Molecules—*unc* Genes and Their Role in Neuronal Function and Development

The first genetic screens in *C. elegans* focused on the most simple and accessible phenotypes, including locomotion capability and size (Brenner, 1974). “Uncoordinated” mutants that were paralyzed or failed to move with the typical sinusoidal waveform were mapped to genes named *unc*. Although many of these genes affect components of muscle, several *unc* genes were later found to be critical for the development and function of neurons.

unc-5 and *unc-40* are receptors for the molecule Netrin, first discovered in *C. elegans*, and encoded by the gene *unc-6* (Hedgecock et al., 1990). These molecules function in axon guidance, the ordered and stereotyped manner of leading axons to their final targets, the processes for which are highly conserved from invertebrates to vertebrates. In *C. elegans,* UNC-6/Netrin is expressed by cells mostly on the ventral side of the animal, and this acts as a guidance cue to attract cells expressing UNC-40/*D*eleted in *C*olorectal *C*ancer (Chan et al., 1996; Wadsworth et al., 1996) but also to repulse cells expressing UNC-5 alone or together with UNC-40/DCC (Leung-Hagesteijn et al., 1992). In addition, UNC-6 and UNC-40 are required in postdevelopmental stages to regulate synaptogenesis, such as in the interneuron AIY, where these molecules are required for the formation of a synapse-rich region between AIY and its postsynaptic partners RIA, AIZ, and RIB (Colón-Ramos et al., 2007). The innexins UNC-7 and UNC-9, which, as discussed, are required for gap junction communication between the command interneurons and the cholinergic locomotion motor neurons, were also identified from these genetic screens.

Several molecules that modulate synaptic function were first discovered via *C. elegans unc* mutant screens. UNC-13/Munc13, UNC-18/Munc18, and UNC-104/KIF1A mutant animals were identified based on a severe paralysis phenotype (Brenner, 1974; Hall & Hedgecock, 1991) and also show strong resistance to acetylcholinesterase inhibitors, indicating a neurotransmission defect (Hosono et al., 1989; Nguyen et al., 1995). However, these mutants display normal nervous system anatomy (Hall & Hedgecock, 1991; Richmond et al., 1999; Weimer et al., 2003). The defects seen in *unc-13* and *unc-18* mutants were shown to be due to the role of these molecules in regulating synaptic vesicle exocytosis and neurotransmitter release, through interactions with the fusion-promoting molecule syntaxin (UNC-64 in *C. elegans*). Although some details remain to be clarified, it appears that UNC-18 promotes docking of vesicles to the plasma membrane (Weimer et al., 2003; McEwen & Kaplan, 2008), whereas UNC-13 later promotes vesicle fusion (Richmond et al., 1999). Additionally, UNC-104 is a kinesin motor that functions in anterograde transport of synaptic vesicles in the nervous system (Hedgecock et al., 1990; Otsuka et al., 1991).

Egg Laying

C. elegans hermaphrodites reproduce by self-fertilization, by producing sperm first and then oocytes, which are fertilized in the spermatheca and stored in the uterus until egg laying takes place. Egg laying occurs when muscles in the egg-laying organ, the vulva, contract, allowing the vulva to open and eggs to be released. These muscles receive input via synapses from the HSN and VC motorneurons (reviewed in Schafer, 2006). Under favorable conditions, the timing of egg-laying events follows a tightly regulated temporal pattern that consists of active states, short bursts of egg laying lasting several (1–2) minutes, which are separated by longer quiescent periods (20 or more minutes) called inactive states (Waggoner, Zhou, Schafer, & Schafer, 1998). Both of these behavioral states can be modeled as exponentially distributed random variables with characteristic rate constants (Zhou, Schafer, & Schafer, 1998). Mutants that show defects in egg laying, termed *egl*, were initially identified in a forward genetic screen for animals bloated with eggs or "bags of worms" where progeny had hatched within the uterus (Trent et al., 1983). These mutants are termed Egl-d, for egg laying defective. Conversely, mutants that show hyperactive egg laying (Egl-c, egg laying constitutive) lay eggs under normally inhibitory conditions (starvation or hypertonic media), lay abnormally early-stage embryos, or retain fewer eggs in the uterus. Egl mutants can be broadly divided into genes that have a general effect on cellular function, such as cell-death pathways or neuromodulation, or genes that affect specifically the function of hermaphrodite-specific neurons of the egg-laying circuit (Schafer, 2006). Some important examples are discussed next.

In males, HSNs undergo programmed cell death, or apoptosis (Sulston & Horvitz, 1977; Sulston et al., 1983). Some of the first identified Egl-d mutants were found to contain *egl-1* gf mutations, where the egg-laying defect was due to forced apoptosis in the HSNs (Trent et al., 1983). Screens for revertants of the *egl-1(gf)* phenotype also identified an *egl-1*loss of function (lf) mutation that suppressed the ectopic programmed cell deaths of the HSNs as well as all normally occurring apoptosis events, indicating that *egl-1* encodes a cell death activator (Conradt & Horvitz, 1998). Programmed cell death is a critical and highly conserved process, and *C. elegans* is a uniquely suitable organism to study the molecular mechanisms involved, due in part to its essentially invariant somatic cell lineage. Some of the most important cell death genes (*ce*ll *d*eath abnormal), *ced-3, ced-4*, and *ced-9*, were discovered in the nematode model and have been shown to function in the same pathway as EGL-1, as double mutants of these genes are non-Egl and have surviving, functional HSNs (Ellis & Horvitz, 1986; Hengartner et al., 1992). All of these genes have functional counterparts in mammals (Metzstein et al., 1998).

Mutations in genes that encode components of GPCR signaling also lead to egg-laying defects. Based on sequence similarity, *C. elegans* appears to express one ortholog of each of the four mammalian G-alpha subunit families, namely GOA-1 (Gi/o), EGL-30 (Gq), GSA-1 (Gs), and GPA-12(G12). The remaining *C. elegans* G-alpha proteins, of a total of 21, are most similar to Gi/o, but they have yet to be classified due to insufficient homology. *goa-1* and *egl-30* are strongly Egl, with *goa-1(lf)* mutants being Egl-c (Ségalat et al., 1995) and *egl-30(lf)* mutants being Egl-d (Brundage et al., 1996; Shyn et al., 2003). *goa-1* and *egl-30* both function within the HSNs and act to negatively and positively regulate HSN

activity, respectively (Shyn et al., 2003). These G-alpha protein subunits are likely to act downstream of multiple GPCRs (Moresco & Koelle, 2004). Furthermore, two of the major enzymes required for neuropeptide processing in *C. elegans* regulate egg laying: After the signal peptide has been cleaved from the prepropeptide to form a propeptide, proprotein convertases like EGL-3/PC2 (proprotein convertase type 2) cleave this molecule into smaller peptides (Kass et al., 2001; Husson et al., 2006) that are then targeted by carboxypeptidases, including the neuron-specific EGL-21 (carboxypeptidase E) for cleavage of basic residues (Jacob & Kaplan, 2003), followed by other posttranslational modifications such as amidation (reviewed in Li & Kim, 2009). Both *egl-3* and *egl-21* mutants are Egl-c, suggesting that neuropeptides processed by these enzymes act to inhibit the egg-laying circuit. Interestingly, *egl-3* and *egl-21(lf)* mutants, despite likely acting in the same pathway, seem to have different overall effects. For *egl-3(lf)* mutants, no peptides could be isolated (Husson et al., 2006), but a low level of FMRFamide-like immunoreactivity was detected (Kass et al., 2001). In contrast, *egl-21* null alleles show more severe defects spanning multiple behaviors compared with *egl-3*, and they have an even lower level of FMRFamide-like immunoreactivity (Jacob & Kaplan, 2003).

Pharyngeal Pumping

C. elegans uses the pharynx, a neuromuscular organ that pumps rhythmically, to suck food (bacteria) from the outside to the intestine. The pharyngeal nervous system consists of 20 neurons, of which three (M3, M4, and MC) appear most important for normal feeding (Avery & Horvitz, 1989; Raizen & Avery, 1994; Raizen et al., 1995), whereas the role of the others remains largely obscure. There are several important ion channels expressed in the pharynx muscle that are required for pumping and peristalsis, including L-type and T-type calcium channels (EGL-19 and CCA-1), a negative spike potassium channel (EXP-2), an acetylcholine receptor (EAT-2), and a glutamate-gated chloride channel (AVR-15) (reviewed in Avery & You, 2012). AVR-15 is of particular importance as this is the target of the nematocidal drug ivermectin. Ivermectin is used not only to control infections in livestock but also in the treatment of debilitating human diseases such as onchocerciasis (river blindness) (Lankas & Gordon, 1989; Campbell, 1993). Although AVR-15 belongs to a family of ligand-gated chloride channels that includes vertebrate GABA-A and glycine receptors, glutamate-gated chloride channels are not found in vertebrates. In fact, the vertebrate ligand-gated chloride channels are also less sensitive to ivermectin than glutamate-gated channels such as AVR-15. Importantly, this allows ivermectin to be relatively harmless to mammals but effective against nematodes and other parasites (Cully et al., 1994; Vassilatis et al., 1997).

Pumping rate is modulated by a range of neuromodulators, including biogenic amines and neuropeptides (Rogers et al., 2001), and here the MC neurons play a central role (Avery & Horvitz, 1989; Raizen et al., 1995). Activated by serotonin (Song & Avery, 2012), these cholinergic neurons phasically stimulate the pharyngeal muscle to determine contraction rate (Trojanowski et al., 2016). Recognition of favorable food (based on previous experience) results in stimulation of the ADF amphid neurons to release serotonin, which then activates the MCs via the receptor SER-7. Interestingly, even a small amount of contamination with novel bacteria interferes with this upregulation, by suppressing either ADF or neurons upstream (Song et al., 2013). Additionally, FMRFamide neuropeptides also potently (i.e., in the nanomolar range) influence pharyngeal activity—neuropeptides that cause increased activity, as recorded by electropharyngeograms (EPGs) include FLP-8, FLP-17A, and FLP-17B, whereas inhibitory neuropeptides include FLP-11 and FLP-13 (Rogers et al., 2001; Papaioannou et al., 2005). Interestingly, FLP-11 and FLP-13 also influence sleep-like behavior in *C. elegans,* as further discussed later.

Defecation

Defecation behavior in *C. elegans* follows a rhythmic and stereotyped motor program (the defecation motor program, or DMP) that consists of three sets of muscle contractions: (1) contraction of posterior body-wall muscles (pBoc), (2) contraction of anterior body-wall muscles (aBoc), and (3) enteric muscle contractions (EMC) that finally results in expulsion of gut contents (Exp) (reviewed in Branicky & Hekimi, 2006). The DMP can be

readily studied as all three sets of contractions can be observed under a dissection microscope. One of the most interesting findings from studies of the DMP is the discovery of the GABA-gated cation channel EXP-1, which provided the first evidence of an ionotropic GABA receptor that acts as an excitatory ligand-gated ion channel that is cation selective (Beg & Jorgensen, 2003), which is surprising because GABA acts in most systems as an inhibitory neurotransmitter (Tobin, 1991) The motor neurons AVL (in the head) and DVB (in the tail) stimulate EXP-1-expressing enteric muscles during the defecation cycle by releasing GABA, causing muscle contraction and expulsion of intestinal contents (McIntire et al., 1993a, 1993b). Therefore, *exp-1(lf)* mutants are defective in excitatory GABA functions such as defecation, but they show normal locomotion and foraging that require inhibitory GABA functions (McIntire et al., 1993a).

Another interesting finding is that inositol-1,4,5-triphosphate (IP_3) (Walker et al., 2002) and the IP_3 receptor ITR-1 (Dal Santo et al., 1999), an ER-localized calcium release channel, play a central role in the ultradian pacemaker of the DMP. The defecation cycle, or the time between two pBocs, is correlated with cyclic fluctuations of calcium ion levels in the intestine, where calcium levels peak in the posterior of the intestine immediately before initiation of the pBoc, and then propagate toward the anterior (Espelt et al., 2005). These oscillations determine the precise timing of the release of signals initiating at least two of the motor events. ITR-1 expression in the intestine is necessary and sufficient for normal DMP rhythms (Dal Santo et al., 1999). Proton release, through the Na+/H+ exchanger PBO-4, from the posterior intestine stimulates contraction of the body wall muscles, and thus pBoc (Beg, Ernstrom, Nix, Davis, & Jorgensen, 2008; Pfeiffer et al., 2008). Release of a neuropeptide-like protein, NLP-40, from the intestine is what triggers AVL and DVB to release GABA, triggering expulsion (Wang et al., 2013). How the intervening aBoc step is triggered is as yet unclear.

C. elegans Behavioral States

Animal behavior tends to fall into distinct, long-lasting, and stable categories termed behavioral states. It is thought that transitioning between behavioral states could either be a response to an external stimulus or is internally generated due to the absence of specific triggers (Flavell et al., 2013). The best-characterized behavioral states include the easily observable sleep/wake cycles, which are mediated by monoamine or neuropeptide neuromodulators. For example, sleep in mammals is mediated by galanin and melanin-concentrating hormone (MCH) (Saper et al., 2010), whereas in *Drosophila melanogaster* this appears to require serotonin (Sehgal & Mignot, 2011). Neuromodulators also regulate behavioral states in *C. elegans*, such as dwelling/roaming states during feeding, cycles of sleep/wake, and arousal.

Foraging

During feeding, *C. elegans* appears to transition between two distinct states: roaming, where animals move across a bacterial lawn with high speed and few turning events, or dwelling, where animals move with low speed and show more frequent turning (Shtonda & Avery, 2006; Ben Arous et al., 2009). The choice between these two states appears to reflect multiple factors: food quantity or quality, metabolic status as regulated by the insulin signaling pathway, or environmental cues detected by ciliated sensory neurons (Fujiwara et al., 2002; Shtonda & Avery, 2006; Ben Arous et al., 2009). Consistent with this, mutants such as *eat-2* and *inx-20* that decrease the pumping rate of pharyngeal muscle, and hence reduce feeding, show less exploratory behavior (Flavell et al., 2013). Serotonin promotes dwelling, as mutations in *tph-1*, the rate-limiting enzyme for serotonin biosynthesis, lead to increased roaming and less dwelling. This pathway acts via the serotonin-gated chloride channel MOD-1 (Flavell et al., 2013). In contrast, pigment dispersing factor (PDF) neuropeptide signaling promotes roaming, as mutations in genes encoding PDF peptides (*pdf-1* and *pdf-2*) or the PDF receptor (*pdfr-1*) lead to prolonged dwelling (Choi et al., 2013; Flavell et al., 2013). Interestingly, defects in PDF signaling also abolish mate searching behavior in *C. elegans* males (Barrios et al., 2012).

Sleep

Sleep was first identified in *C. elegans* as a quiescent behavioral state that occurs during defined periods of development. These periods, called lethargus, occur before each of the four larval moults and correlate with oscillations of the worm Period homolog LIN-42 (Jeon et al., 1999). Quiescence associated with *C. elegans* lethargus also displays sleep-like properties consistent with other animals, such as homeostasis, reversibility, and reduced sensory responsiveness (Raizen et al., 2008). One of the first identified genetic regulators of lethargus is EGL-4 (cGMP-dependent protein kinase), which promotes quiescence behavior: gf mutants of *egl-4* show quiescence in normally active periods, whereas lf mutants have reduced behavioral quiescence during lethargus (Raizen et al., 2008). Interestingly, *egl-4(lf)* mutants also show increased exploratory behavior during feeding (Fujiwara et al., 2002; Flavell et al., 2013). In addition, neuropeptides have been shown to regulate sleep behavior. FLP-11 released from the RIS neuron (Turek et al., 2016) and NLP-22 released from the RIA interneurons (Nelson et al., 2013) both promote the sleep state during lethargus. For FLP-11-dependent sleep, multiple neuropeptide receptors, including NPR-4, NPR-22, and FRPR-3, in addition to other unknown receptors, are required to modulate this behavior (Turek et al., 2016). Consistent with the important role of GPCRs in regulating quiescence during lethargus, the *C. elegans* G protein alpha subunit Gq *egl-30* also promotes sleep (Schwarz & Bringmann, 2013). Another sleep-like behavior in *C. elegans* is defined as stress-induced sleep, in which animals become behaviorally quiescent following cellular stress, such as following heat exposure. Induction of this behavioral state requires the neuron ALA and release of FLP-13 neuropeptides from this neuron (Nelson et al., 2014). These peptides bind the receptor

DMSR-1 and act to promote sleep, potentially by inhibiting the activity of wake-promoting neurons (Iannacone et al., 2017).

Arousal

On the other side of the spectrum from sleep is the behavioral state known as arousal. In response to stimuli associated with danger, animals undergo a sustained state of hypervigilance that generates increased locomotor activity and sensory responsiveness (Choi et al., 2015). For example, *C. elegans* becomes aroused in response to high oxygen concentration, displaying high locomotory speed and turning behavior at 21% oxygen levels (Gray et al., 2004; Busch et al., 2012). These responses are observed specifically in strains carrying the naturally occurring allele of the *npr-1* neuropeptide receptor mutant background, as the reference laboratory strain N2 has acquired an *npr-1(gf)* mutation during laboratory cultivation (McGrath et al., 2009) that inhibits some oxygen responses (de Bono & Bargmann, 1998). The oxygen-sensing neurons of *C. elegans*, AQR, PQR and URX, are tonic receptors that continuously detect ambient oxygen concentration and set the animal's behavioral state accordingly. For example, *npr-1* animals move rapidly on food at 21% oxygen levels and sustain this speed for 2 hours. In contrast, at 7% oxygen levels these animals show reduced locomotion speed and increased dwelling, also for sustained periods. However, transitioning between these long-lasting behavioral states can occur rapidly (within seconds) in response to switches in oxygen concentration, demonstrating that *C. elegans* is able to change its behavioral state persistently in response to ambient oxygen (Busch et al., 2012). Interestingly, *npr-1* also alters locomotion quiescence during lethargus—*npr-1* deletion mutants and mutants lacking NPR-1 ligands, FLP-18 and FLP-21, are hyperactive during lethargus (Choi et al., 2013). Many of the effects of NPR-1 on foraging and "social" (i.e., aggregation-prone) behaviors are dependent on its expression in the motor/interneuron RMG, which forms gap junctions with multiple sensory neurons (forming an "RMG circuit"), where high RMG activity is correlated with social behavior (Macosko et al., 2009). High NPR-1 activity inhibits RMG in "solitary" strains such as reference strain N2, but "social" wild *C. elegans* isolates have *npr-1* alleles that confer low activity and are correlated with aggregation and high RMG activity (de Bono & Bargmann, 1998; Macosko et al., 2009). Consistent with this, the defects in locomotion quiescence in *npr-1* mutants also appear to be dependent on heightened activity in the RMG circuit, as well as mechanosensory and stretch-sensing neurons (Choi et al., 2013, 2015).

Closing Remarks

The small number of neurons possessed by *C. elegans* belies surprisingly sophisticated processing power and, indeed, a connectome of unexpected complexity. The constraints of such a small nervous system may require unique connectome adaptations. An example

is the multifunctional nature of sensory neurons, such as the expression of multiple olfactory receptors in a single neuron, as opposed to the single receptor type per neuron in flies and vertebrates. Nevertheless, the mechanisms employed for sensory processing, motor control, and plasticity illustrate many common themes seen in higher organisms, and they have provided significant insight into how these function. Despite a wealth of information from the mapping of the worm connectome, and both ablation and genetic studies that have pinpointed the roles of individual neurons, many questions remain unanswered. As we have discussed, some exciting recent advances have helped us to understand precisely how sensory information is integrated and translated into a motor response and, importantly, how experience and behavioral state act on the molecular components, and thus the circuit, to impact on these.

Many *C. elegans* genes are extremely highly conserved in mammals. As we have seen, *C. elegans* forward genetics, in particular, has played an important role in identifying founder members of gene families, thus elucidating the molecular basis of nervous system development and function. Its ease of cultivation, genetic tractability, and the wide range of well-characterized phenotypes and behaviors mean that it represents a powerful model in which to piece together components of pathways, and to understand dysfunction and disease in the nervous system. This, combined with the amenability to high-throughput approaches, and the ever-expanding technological toolbox, means that *C. elegans* research also has huge potential to contribute to the identification of new therapeutic approaches and molecules.

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