

## Hypothesis

Using neurons to maintain autonomy: Learning from *C. elegans*William Bechtel<sup>a,\*</sup>, Leonardo Bich<sup>b</sup><sup>a</sup> Department of Philosophy; University of California, San Diego; La Jolla, CA 92093-0119, USA<sup>b</sup> IAS-Research Centre for Life, Mind and Society; Department of Philosophy; University of the Basque Country (UPV/EHU); Avenida de Tolosa 70; Donostia-San Sebastian, 20018; Spain

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## ABSTRACT

Understanding how biological organisms are autonomous—maintain themselves far from equilibrium through their own activities—requires understanding how they regulate those activities. In multicellular animals, such control can be exercised either via endocrine signaling through the vasculature or via neurons. In *C. elegans* this control is exercised by a well-delineated relatively small but distributed nervous system that relies on both chemical and electric transmission of signals. This system provides resources to integrate information from multiple sources as needed to maintain the organism. Especially important for the exercise of neural control are neuromodulators, which we present as setting agendas for control through more traditional electrical signaling. To illustrate how the *C. elegans* nervous system integrates multiple sources of information in controlling activities important for autonomy, we focus on feeding behavior and responses to adverse conditions. We conclude by considering how a distributed nervous system without a centralized controller is nonetheless adequate for autonomy.

## 1. Introduction

Biological organisms are autonomous systems—they maintain themselves far from equilibrium through the action of a host of productive processes (mechanisms) that take in resources and process them to build and repair themselves and perform actions in the world to, among other things, defend themselves and procure resources. This understanding of biological organisms has been investigated by researchers in what is now known as the “biological autonomy”<sup>1</sup> research tradition. The tradition is rooted in the theorizing of Claude Bernard (1865) and of Cybernetics and Systems Theory and built upon the contributions of numerous theorists in the late 60s and early 70s including Jean Piaget (1967), Robert Rosen (1972), Humberto Maturana and Francisco Varela (Varela et al., 1974), Howard Pattee (1972) and Tibor Gánti (1975).<sup>2</sup> In recent years it was further developed by Stuart Kauffman (2000), Alvaro Moreno and his collaborators (Ruiz-Mirazo and Moreno, 2004; Moreno and Mossio, 2015), and others. Theorists in this tradition emphasize that organisms are capable of constructing, repairing, and maintaining their parts, and consequently themselves,

through the continuous exchange of matter and energy with the environment. To explain this capacity, this tradition appeals to the internal organization of the organism, the way production and transformation processes are connected so that they are able to synthesize the very components that subsequently constitute them by using energy and matter from the environment. This organization is what is maintained despite the continuous transformations that the organism undergoes at the level of its components.

Recent contributions to this tradition have emphasized that to maintain themselves, autonomous systems need to be organized so to be able to control the activities of their components (Bich et al., 2016; Winning and Bechtel, 2018; Bich and Bechtel, 2022b). Components responsible for productive processes must be regulated so that their activities are performed when and in the manner needed. In single-cell organisms, most of the control is carried out through chemical signaling within the organism or between organisms.<sup>3</sup> Such control processes remain critical for multicellular organisms, but multicellular organisms confront an additional challenge—controlling, across longer distances, the activities of individual cells and of groups of cells that

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constitute their tissues and organs, so that they carry out the activities required by the whole organism. To do this, multicellular organisms adapt two strategies already manifest in single-cell organisms—the diffusion of chemical signals in the extracellular milieu and conduction of ion changes along the membrane of cells.<sup>4</sup> To enable long distance communication of chemical signals in multicellular organisms unconstrained diffusion is not enough; organisms require a vasculature (xylem in plants, circulatory systems in many animals) (Bich et al., 2019). The nematode worm *Caenorhabditis elegans* lacks a circulatory system. Hence, it relies primarily on electrical transmission by specialized cells, neurons, to coordinate the activity of its nearly 1000 cells.<sup>5</sup> A nervous system can provide for fast communication between distant parts of the system and potentially affect a high number of cells. Given its well delineated and relatively simple nervous system *C. elegans* provides an excellent model organism for understanding how nervous systems can support autonomy in multicellular organisms and shed light on general features of biological control.

The adult *C. elegans* (hereafter, the worm) is a translucent free-living round worm or nematode about 1 mm in length that lives on lawns of bacteria in rotting vegetable matter. It is an extensively studied model organism (for a detailed discussion of what constitutes a model organism and how they are used in biology, see Ankeny and Leonelli, 2020). It goes through four larval stages over three days and then lives for approximately 20 days. The laboratory strain or wildtype, N2, was isolated from a mushroom compost heap in Bristol, England in the 1960s (Dallière, Holden-Dye, Dillon, O'Connor and Walker, 2017).<sup>6</sup> N2 is commonly raised exclusively on *Escherichia coli* OP50, but in the wild worms encounter multiple species of bacteria, amongst which they select some to ingest through pharyngeal pumping. Of the bacteria they ingest, worms digest some to provide energy and materials from which to synthesize their own bodies and sustain their activities; others they incorporate into their gut microbiome. Not all bacteria are nutritious for worms; some are pathogenic (Kim and Flavell, 2020). Worms try to avoid these, but if they ingest them, they mount an immunological response. Many of the activities worms perform to maintain themselves involve coordinating their lives with bacteria.

Most worms are hermaphrodites—i.e., organisms capable of fertilizing their own eggs. Males constitute only a minute proportion (<0.02%) of worm populations; accordingly, we limit our discussion to hermaphrodite worms. Each hermaphrodite contains 959 cells, of which 143 are muscle cells and 302 are neurons. These cells fall into identifiable types that can be reliably reidentified from worm to worm, facilitating systematic study of the mechanisms operating in the species. Neurons play a central role in regulating behavioral mechanisms such as those involved in locomotion, feeding, defecation, and egg-laying. The

sensory capacities of worms include photosensation, chemosensation, mechanosensation, osmosensation, thermosensation as well as proprioception (Ilf and Xu, 2020).

A factor that makes examination of neural control in the worm promising is that, due to the efforts of White et al. (1986), who used serial electron microscopy to trace the approximately 7000 projections between the worm's 302 neurons, researchers have a nearly complete connectome that provides a major reference point for analyzing its neural processing.

Several other factors render the worm a valuable model for understanding how a nervous system enables the control needed for organisms to be autonomous. Among them, three are particularly relevant. (1) The worm lacks a central brain. When organisms have a brain, and especially a highly evolved brain, there is a tendency of researchers to concentrate on the brain and its “highest” centers. However, even in organisms with a brain, much of the nervous system is decentralized, and in the worm it is fully decentralized. This facilitates investigating how a highly distributed system can nonetheless enable the worm to integrate the activities of its parts and function autonomously. (2) The worm's nervous system is grounded in the physiological processes carried out by the other cells of its body. Unlike an electronic computer, the worm's neurons are highly integrated with the chemical processes they control. (3) This connects with a third feature: the nervous system in the worm is not a purely electrical system, but an electrochemical system that makes extensive use of neuromodulators. Neuromodulators operate differently than glutamate and GABA that serve to extend electrical activity from a presynaptic neuron to a postsynaptic one as a result of being released at a synapse and acting specifically on ionotropic receptors of the postsynaptic neurons (and otherwise degraded). Neuromodulators (typically monoamines and neuropeptides<sup>7</sup>) are secreted from diverse locations on neurons, diffuse through the extracellular matrix (in worms, through the pseudocoelomic cavity, a fluid-filled body cavity between the internal organs and the external body wall), and act on any neuron with appropriate metabotropic receptors. In most cases these receptors are G-protein coupled receptors (GPCRs) that elicit second messengers within the cell that change its metabolism, often initiating new gene expression. These effects are much longer lasting than those that suffice to elicit electrical activity in a post-synaptic neuron; we will characterize them below as setting the agenda for other neural processing. Targeted signaling acting on ionotropic receptors then enables the execution of the agenda.

By drawing upon these features of the worm, we seek to advance an understanding of basic principles of how neurons support autonomy in animals, features that are conserved in higher animals such as humans but are often not appreciated. We begin in section 2 with neuro-modulation and its role in agenda setting. Subsequent sections focus on how setting and executing agendas enable the worm to control those activities necessary to maintain autonomy. In section 3 we turn to a critical activity for any animal—finding nutrients. Befitting its importance, a major portion of the nervous system in worms (and in us) is directed to coordinating not just the procurement of nutrients but also their ingestion and digestion. No animal, though, can be exclusively focused on procuring nutrition; animals must detect, evaluate, and respond to threats. Avoiding threats altogether is not an option if a worm is to carry out the activities required to maintain itself—these activities expose the worm to predation and other threats. Accordingly, the worm must control its responses to adverse conditions appropriately to reduce the risk of injury and illness while pursuing the activities it must perform to remain alive. In section 4 we analyze the control mechanisms through which worms selectively respond to aversive stimuli. In the final section we discuss the implications of this analysis for a more general

<sup>4</sup> On electrical communication in bacterial biofilms, see Prindle et al. (2015).

<sup>5</sup> By focusing on control between cells, we will not examine intracellular control. *C. elegans* employs many such intracellular mechanisms. For example, what is referred to as the proteostasis network is large and generally suffices (albeit with less success as the worm ages) to maintain the proteins of a cell in conditions in which they can carry out their activities (Zhang et al., 2022; Hoppe and Cohen, 2020). Moreover, some control of cells by other cells is carried out chemically through the secretion and response to endocrines. Without a vasculature, diffusion cannot be directed over long distances. Chemicals (ascaroside pheromones) also figure in coordination of activities between worms (Muirhead and Srinivasan, 2020). By focusing on neurons, we are providing only a partial account of the mechanisms through which worms control their activities.

<sup>6</sup> The strain has undergone genetic changes that are adaptations to laboratory conditions. One dramatic change is that it has altered its oxygen preference for approximately 8% oxygen to tolerate the approximately 21% oxygen level in ambient air in the laboratory by modifying the *npr-1* gene. The N2 strain also exhibits differences from other strains found in the wild in terms of tolerance for CO<sub>2</sub>, aggregation when feeding, and roaming versus dwelling locomotion on bacterial lawns (Kim and Flavell, 2020).

<sup>7</sup> As with many generalizations, there are exceptions. In some cases, glutamate and GABA operate like neuromodulators and some monoamines and neuropeptides act like classical neurotransmitters.

understanding of biological control, and in particular the importance and reach of the idea of agenda setting beyond this case study.

## 2. Neuromodulators: setting the agenda for electrical processing by neurons

When White and his collaborators developed the connectome for the worm, nervous systems were generally viewed as electrical switching systems. Thus, one could hope to use the connectome to trace how electrical signals would be processed. Moreover, the system was envisaged as a feedforward processing system from sensory neurons to motor neurons, with those that were neither sensory nor motor functioning as interneurons that conveyed activity from sensory neurons to motor neurons. The same researchers who developed the connectome offered a model of how one could utilize it to explicate neural processing. Chalfie et al. (1985) proposed a circuit through which a worm reverses its forward motion when touched at the front (Fig. 1, left). Touch to a worm's head activates AVM and ALM sensory neurons whereas touch to the tail activates PLM neurons. These sensory neurons project to four interneurons either via synapses or through gap junctions (connections between the cytoplasm of the two neurons that enable electrical activity to extend from one neuron to another). On their account, when activated, the four interneurons activate motor neurons, which generate either forward or backwards movement. The anatomical studies on which the connectome was based did not reveal whether connections are excitatory or inhibitory. Nonetheless, one can readily envisage how activation of the AVM and ALM head neurons would, through gap junctions to the interneuron AVD, activate VA/DA motor neurons to initiate backwards movement directly or via its projections to the interneuron AVA (a similar account suggests how activating PLM results in forward movement).

This and other accounts based on the connectome are suggestive, but subsequent research has shown that while these circuits are anatomically fixed, how they process information can change. How are these circuits modulated, and which are the controllers responsible for that? Working not in the worm, but in the stomatogastric ganglion of the lobster, for which researchers had likewise developed a detailed circuit map, Marder discovered that when a neurotransmitter such as dopamine was applied to the circuit, several neurons changed how they functioned (for an overview, see Marder, 2012). Without altering the connectivity, the same circuit would process information in different ways. Bargmann generated similar results after applying either monoamines or neuropeptides in the worm (for an overview of her research see Bargmann, 2012; Bargmann and Marder, 2013 review work in both the worm and the lobster). Because these neurotransmitters modulate how circuits behave, they came to be known as neuromodulators (Katz, 1999).

Neuromodulators in fact affect the circuit described by Chalfie et al., enabling the worm to exhibit more complex behaviors. As described by Donnelly et al. (2013), a worm normally moves forward by propagating a sinusoidal wave of muscle contractions along its body, accompanied by moving the tip of its nose rapidly from side to side. When its head is touched, it stops moving its head and bends deeply so that its head moves alongside its body and eventually touches its tail. The worm then straightens and resumes sinusoidally movement, but now in the opposite direction. This adjustment takes about 10 s and requires several neurons in addition to those identified by Chalfie et al. The RIM neurons (shown in tan in Fig. 1, Right) explains several of these effects. RIM releases both traditional neural transmitters and the neuromodulatory monoamine tyramine (it is in fact common for neurons to release multiple transmitters). Through acting on the ionotropic inhibitory chloride channel in RIV and SMD motor neurons (not shown), which control neck and head movements, the RIM neurons inhibit head movements. Second, RIM acts in an inhibitory fashion on an ionotropic receptor on AVB to inhibit forward movement. Third, through release of tyramine, it acts on the GPCR SER-2 (also in tan) in VD neurons, causing these neurons to release GABA, which inhibits ventral wall bending. The prolonged

response of the GPCR accounts for the sustained inhibition while the worm reorients. Donnelly et al. (2013) argue that the inclusion of tyramine in the process is crucial for generating the coordinated extended turning movement of the worm.

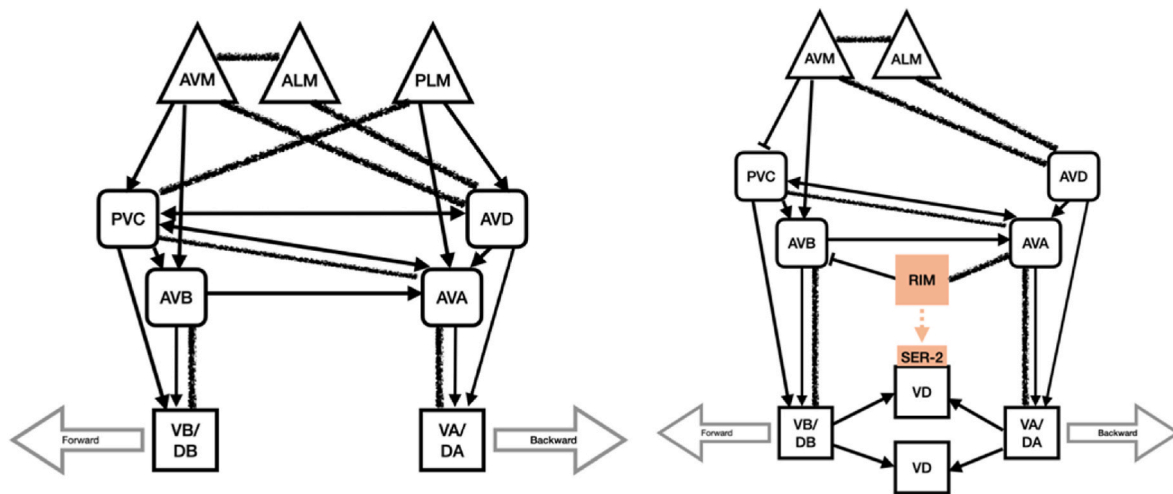
Traditional accounts of behavior, such as that advanced by Chalfie et al., emphasize how an organism responds to external stimuli. However, for organisms to deploy their capacities to carry out activities in ways that serve the needs of the organism to maintain itself, they need to “integrate changes in their external environments with their internal states (Alcedo and Prahlad, 2020).” The diffuse and slow response to neuromodulators makes them well suited for registering enduring conditions in the organism, disseminating that information, and using it to modulate the electrical activity of neurons over a sustained period of time (Flavell et al., 2013). By coordinating this with the relatively fast response of classical transmitters, organisms can perform the integration Alcedo and Prahlad reference.

Given their role in coordinating responses to the state of the organism over longer timescales than electrical signaling, the characterization of monoamines and neuropeptides as modulators does not do justice to their role in the operation of the nervous system. By registering overall conditions in the organism and its environment they establish enduring configurations of the circuits that respond to more transient inputs. One might better characterize them as using the state of the organism to set the agenda for processing sensory information (Bechtel, 2022).<sup>8</sup>

In the remainder of this section we briefly introduce some of the monoamines and neuropeptides that function as neuromodulators and enable the worm to set the agenda for other neural processing; in subsequent sections we will provide specific examples of how they function in this capacity and contribute to the regulation of those activities necessary to maintain the worm. Monoamines are relatively small molecules synthesized by a series of enzymatic reactions from phenylalanine. Four monoamines—octopamine, tyramine, serotonin, and dopamine—perform important neuromodulatory functions in the worm. (Octopamine and tyramine are structurally quite similar to the vertebrate monoamines epinephrine and norepinephrine, respectively). Each monoamine is synthesized in a small number of neurons from which it diffuses widely. Tyramine is synthesized from tyrosine in RIM motor neurons, while octopamine is in turn synthesized from tyramine in RIC interneurons; dopamine is synthesized from l-dopa (synthesized in turn from tyrosine) in the ADE, CEP, and PDE neurons while serotonin is synthesized from dopamine in NSM, ADF, and HSN neurons. Altogether, only 18 of the 302 neurons in the adult hermaphrodite release monoamines. Yet, 251 or 83% of the worm's neurons have receptors for these transmitters, indicating that they have wide-ranging effects. In addition to their individual effects, monoamines often act pleiotropically with each other and with neuropeptides, generating complex effects.

While only four monoamines function as neuromodulators in the worm, there are a multitude of neuropeptides (more than 120 genes in the worm encode precursors to neuropeptides and more than 250

<sup>8</sup> This point is clearly expressed by Alcedo et al. (2020, p. 475): “An animal receives multiple environmental stimuli, some of which have the potential to disrupt metabolism and overall physiology. To survive environmental stressors, an animal must transition between a range of internal states and behaviors to identify new set points at which its physiological processes function optimally, thereby regaining homeostasis. One mechanism by which all organisms, including *Caenorhabditis elegans*, integrate changes in their external environments with their internal states is through the secretion of chemicals known as neuromodulators, which allow the animal to best exploit its niche and prioritize survival.” Alcedo et al. also emphasize how neuromodulators can enable cells to respond to different conditions in their local environment: “To add to their complexity, a neuromodulator may promote one response by enhancing one cell's activity and/or repressing the activity of another. Then, due to a change in local cell environments, that same neuromodulator may promote a second or opposite response by affecting the activities of other cells that now express the appropriate receptors.”



**Fig. 1.** Left. Circuit proposed by Chalfie et al. to explain how touch regulates backwards and forwards movement. Thickened lines indicate gap junctions, arrows chemical synapses. The connectome did not reveal which chemical transmission is excitatory or inhibitory. Right. Additions of neuromodulatory components to the circuit by Donnelly et al. In this and subsequent figures, neuromodulators will be shown in color. The tyramineric neuron and the GPCR receptor for tyramine are shown in tan and the connection between them with a dashed arrow. B also shows that the chemical projection between AVM and PVC had been shown to be inhibitory.

neuropeptides have been identified). Over 150 GPCRs that respond to neuropeptides have been identified (for a review, see [Borghgraef et al., 2018](#)). A prominent class of neuropeptides are FMRFamide-like peptides (FLPs). To illustrate the roles played by neuropeptides in regulating behavior, consider the role FLPs play in regulating sleep. Sleep, defined behaviorally in terms of quiescence, reduced responsiveness to mild stimulation but maintained responsiveness to strong stimulation, and increased sleep pressure when sleep is prevented or interrupted, is widespread among animals with neurons.<sup>9</sup> In worms it was initially only identified as occurring during development, between each of four larval stages and in the transition to adulthood, but not during adulthood ([Raizen et al., 2008](#)). Here we focus just on larval sleep, known as *lethargus*, returning below to discuss more recent research showing that adult worms also sleep. Sleep bouts in *lethargus* are typically about 3 h, during which feeding and locomotion ceases and worms undergo a number of morphological and developmental changes ([Trojanowski and Raizen, 2016](#)).<sup>10</sup>

Sleep in worms is triggered by a single neuron, RIS. RIS produces both the neurotransmitter GABA and the FLP-11 neuropeptides (four peptides synthesized from the same gene). Because its effects are usually

<sup>9</sup> More recently, microfluid chambers have been used to examine sleep transitions, revealing that adult worms make spontaneous transitions to micro-sleep states ([Gonzales et al., 2019](#)). These micro-sleep states also exhibit a homeostatic response—worms exhibit more micro-sleep bounds after extended wakefulness. In this micro-sleep bouts worms not only exhibit quiescence that is more easily reversible, but adopt a stereotypic posture that is the opposite of developmental sleep—greater curvature.

<sup>10</sup> For a detailed review of sleep in worms, see [Moosavi and Hatam \(2018\)](#). Although sleep is often associated with circadian rhythms, the worm provides a clear instance in which they are distinct. Adult worms do control a number of activities in a circadian manner, including pharyngeal pumping, feeding, defecation, olfaction, stress responses, and expression of many genes, but not sleep ([Migliori et al., 2023](#)). Research has also established that a number of peptides, including PDF and melatonin, are expressed in a circadian manner and in turn regulate daily activities. At present the complement of genes/proteins involved in generating circadian rhythms in the worm is not known, but worms possess homologs of genes whose proteins are involved in generating circadian rhythms in other animals. Many of these also figure in development. One that appears central is *lin-42*, a homolog of the core clock gene *period* found in flies and vertebrates. Like *period* mutants, *lin-42* mutants exhibit disrupted oscillations in molting behavior.

inhibitory, GABAergic activity of RIS was thought to play a critical role in suppressing locomotor activity in sleep. [Turek et al. \(2016\)](#), however, demonstrated that GABA is not, in fact, responsible for engendering the activity suppression found in sleep. Instead, they identified FLP-11 as playing this role and identified three receptors for FLP-11 that are active in different cells involved in control or execution of locomotor activities and to which, with one exception, RIS does not have a synaptic connection: FRPR-3, found in approximately 30 neurons, mostly in the head; NPR-4, expressed, in five neurons; and NPR-22, found in several neurons as well as muscle tissue in the head and pharynx.

Neuropeptides are important as well in activating RIS. [Maluck et al. \(2020\)](#) identified several neurons that activated RIS through glutamatergic synapses, but found that PVC played a crucial role ([Fig. 2](#)). This finding was initially puzzling, since PVC was known to promote forward movement by activating AVB (also shown in [Fig. 1](#)). Further investigation showed that the combined action of PVC and RIM were needed to induce sleep. RIM, as we note above, releases tyramine, for which RIS has a receptor. PVC is one of the neurons acted on by FLP-11 when it is released by RIS. In most situations, PVC and RIM act as a switch so that only one is active and the worm moves either forward or backwards. At the transition between these activities, both are active, producing a weak activation of RIS. When the influence of PVC is especially strong, though, this transitional state can induce strong activation of RIS, enhancing positive feedback through AVE and PVC that maintains RIS activity.<sup>11</sup> Maluck et al. emphasize how this circuit can account for switching. The role of neuropeptides explains how, once the worm switches from waking to sleeping, the state endures for a prolonged time unless a strong stimulus leads to a demand for action. The enduring effects of neuromodulators help maintain these states.

Sleep is an example of agenda setting: the establishment of a sustained regime as a result of the action of neuromodulators (neuropeptides) that coordinate the activity of a larger set of neurons. One way to appreciate the impact of neuromodulators on the overall neural system is to create a network representation comparable to that of the connectome in which neurons are nodes and directed edges (arrows) connect nodes for neurons that release neuromodulators to those for

<sup>11</sup> This relation between pausing the two locomotor circuits and sleep leads Maluck et al. to hypothesize “sleep and sleep-active neurons evolved from systems controlling locomotion activity and rest.”

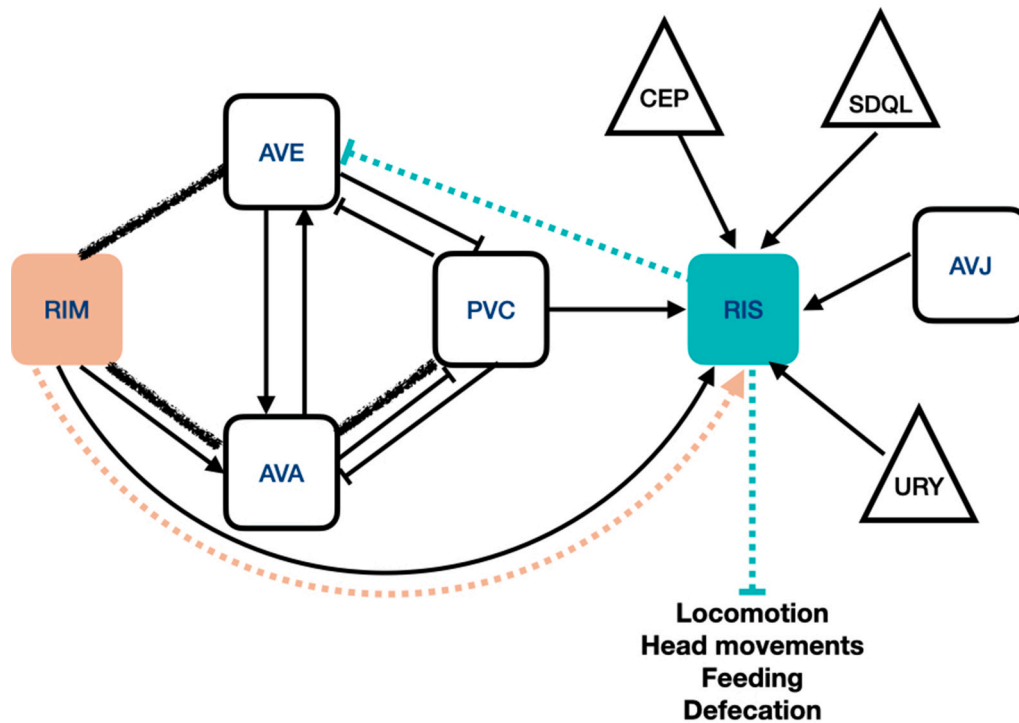


Fig. 2. Network controlling sleep. RIS promotes sleep through the release of the neuropeptide FLP-11, here shown only as acting on the AVE neurons. The interaction of the release of FLP-11 by RIS and of tyramine by RIM play critical roles in inducing and maintaining waking and sleeping states.

neurons with receptors for them. Bentley et al. (2016) constructed separate networks for monoamines and neuropeptides. The network for monoamines included 18 neurons releasing monoamines and 251 neurons with receptors for them. The network exhibited a star-like topology, with a small number of highly connected hubs each projecting to a diverse group of other neurons. In developing a network for neuropeptides, Bentley et al. faced the limitations that, for many neuropeptides, either the releasing neurons or those with receptors are unknown. Accordingly, they included only 12 neuropeptides. The network represented 239 neurons and over 7000 connections. Instead of a star-like topology, this network manifested many clusters. The difference in topology suggests that monoamines and neuropeptides might be performing different functions. Both networks, though, exhibited a rich-club structure, in which the most highly connected nodes are themselves highly interconnected, suggesting each constitutes a highly interconnected system.

Bentley et al. further investigated how these networks, which on our account are responsible for setting agendas, relate to the connectome network of synaptic connections responsible for the execution of agendas. Overall, there is little overlap between the neuromodulator networks or between either of them and the connectome based on synapses, suggesting that each complements the others. However, there are clear points of contact. As shown in Fig. 3, the rich-club networks of both the monoaminergic and synaptic networks are themselves highly interconnected. Half of the neurons in the synaptic rich club have receptors for monoamines, and all the neurons in the monoamine rich club have synaptic connections to those in the synaptic network rich club. While revealing a great deal of integration, this network representation unfortunately does not capture the differences in time and spatial scales at which neurons interact in the two networks. Nonetheless, the monoamine network reveals an important layer of connectivity beyond that revealed in the connectome, supporting Bargmann's (2012) contention that researchers need to look beyond the structural connectome and consider the neuromodulators if they are to understand how the nervous system of worms enables them to maintain themselves. Since many of the neurons that figure in these three networks play

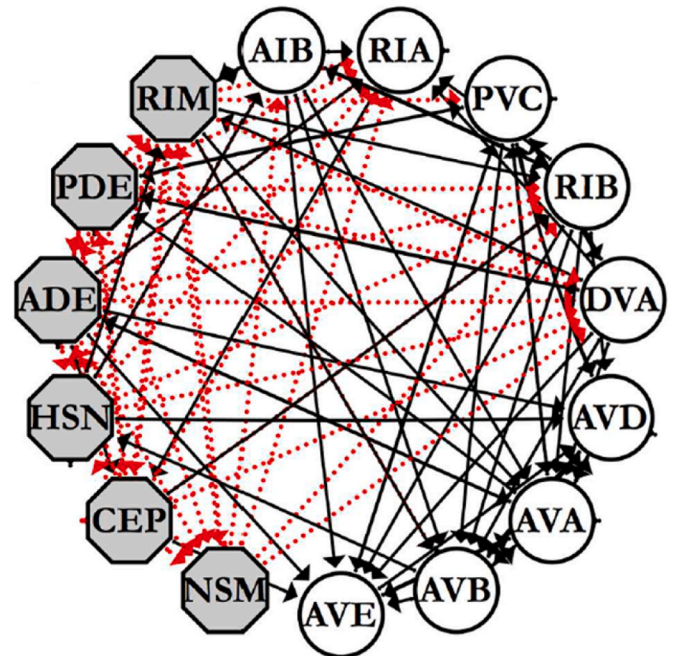


Fig. 3. Connections between neurons in the aminergic rich-club (grey octagons) and wired rich-club (open circles). Reprinted from Bentley et al. (2016). Solid black arrows represent wired connections, red dotted arrows connections of monoaminergic releasing neurons and neurons with receptors for that monoamine.

important roles in the circuits discussed elsewhere in this paper, it is important to keep in mind how interconnected the whole system is.

In this section we have foregrounded the role of neuromodulators in setting the agenda for processing different sources of information. Below we will illustrate their role in registering specific states of the organism

and in enabling the worm to regulate particular activities.

### 3. Adaptively regulating feeding

To maintain themselves far from equilibrium, organisms need to procure food. But both the availability of food and how immediately organisms need it varies. Accordingly, organisms adaptively regulate feeding. In this section we discuss how worms regulate the basic processes through which they procure and consume food. On the one hand, these illustrate how monoamines are employed to set agendas by generating enduring states in which worms carry out the activities relevant to feeding and to switch between these states by reconfiguring the neural circuits responsible for them. On the other, they also reveal how regulation of feeding interacts with other regulatory activities. The result is a highly distributed control network, capable of integrating information from several sources about the current state of the worm and its environment.

Worms are commonly classified as engaging in three activities: roaming, dwelling, or quiescence. Since in laboratories worms are commonly raised on *E. coli*, which for them is a suboptimal nutrient (Ben Arous, Laffont and Chatenay, 2009), they spend little time quiescent. In the wild, however, worms become satiated, and when they do, they cease both roaming and dwelling and enter a quiescent state that resembles sleep.<sup>12</sup> Here we focus on just roaming and dwelling, states worms can maintain for tens of minutes before quickly transiting to the other (Ji et al., 2021). While roaming, worms exhibit prolonged, fast forward movements punctuated with infrequent reversals. In contrast, while dwelling, during which they feed, defecate, and lay eggs, worms move forward slowly and frequently reverse their direction. Feeding involves first taking bacteria into their mouths and then grinding them in their pharynx, a neuromuscular tube 100  $\mu\text{m}$  in length and 20  $\mu\text{m}$  in diameter. Grinding is achieved through a pumping action of the pharynx. Food is then passed to the intestine, where nutrients are absorbed.

Movement and digestion are two alternative enduring regimes. They involve neural control over muscles, but in turn require further control to coordinate with each other. The enduring states of roaming and dwelling are primarily regulated by monoamines, with receptors for them translating registered conditions into appropriate action. Octopamine, released by the RIC neurons, is the primary neuromodulator registering starvation. As shown in Fig. 4, two GPCRs on SIA neurons, SER-3 and SER-6, respond to octopamine to promote increased locomotion (roaming).<sup>13</sup> In contrast, NSM neurons release serotonin when

food is detected. (These neurons are located near the vulva and extend a sensory dendrite into the pharynx to detect the presence of food.) By acting on the MOD-5 receptor on AIY neurons, which normally promotes locomotion, serotonin acts to reduce locomotion. NSM and RIC neurons also each inhibit the other, with NSM acting on RIC via the MOD-1 receptor.<sup>14</sup> NSM neurons also forms a mutual inhibitory circuit with RIM neurons—NSM neurons have a SER-2 tyrosine receptor and so are inhibited when RIM neurons are active while RIM neurons, like RIC neurons, have a MOD-1 chloride-channel ionotropic receptor that binds serotonin. Through their release of serotonin, NSM neurons act on SER-7 receptors on MC, M2, M3, and M4 neurons to promote pumping of the pharynx (Ishita et al., 2020). A consequence is that when RIM and RIC neurons inhibit NSM neurons, they also suppress pharyngeal pumping. ADF neurons also release serotonin in the presence of food that acts on the SER-7 receptors to increase pumping and on SER-5 receptors on various muscles and head movement neurons.

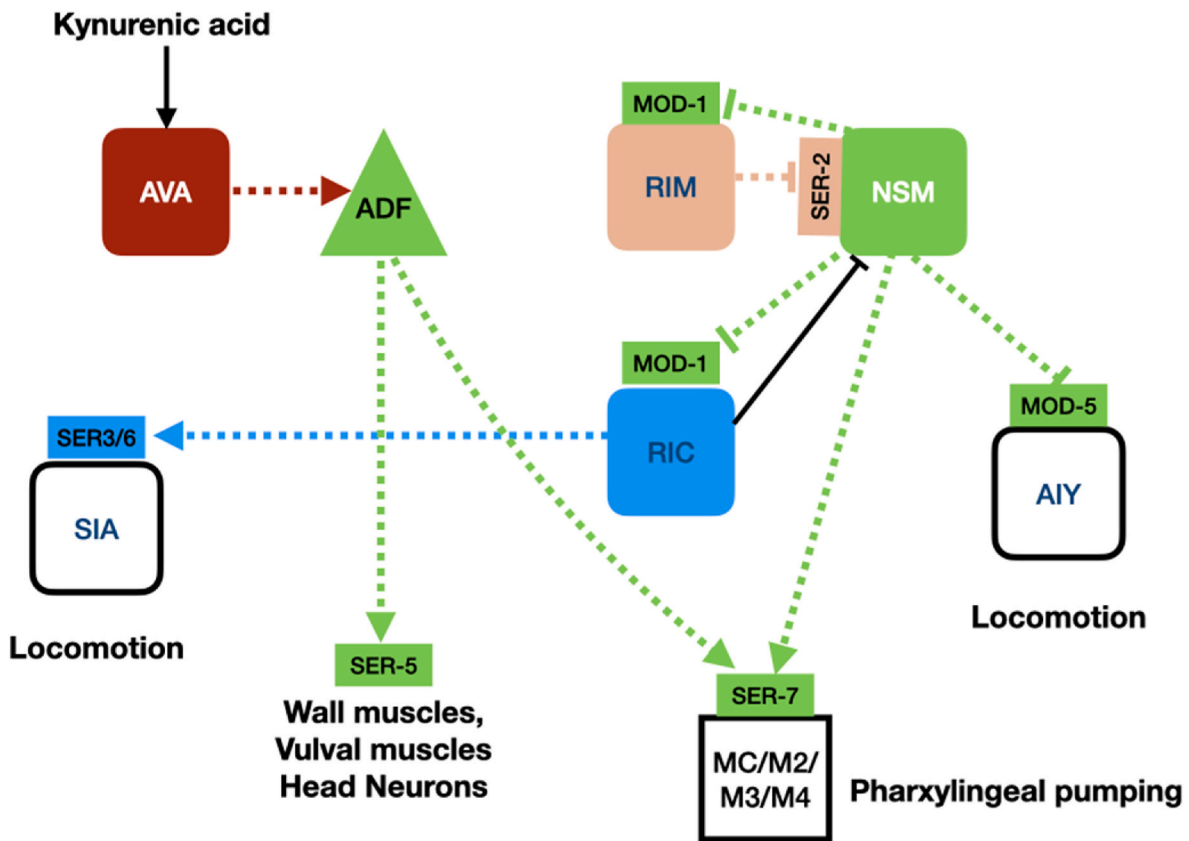
An interesting finding is that worms that encounter food after starvation exhibit faster pharyngeal pumping than do well-fed worms. This is mediated by kynurenic acid, which, like serotonin, is a product of tryptophan and is absent in starved worms. Kynurenic acid usually inhibits the release of the neuropeptide FLP-18 from AVA neurons (shown in red); when kynurenic acid is absent, more FLP-18 is released. FLP-18 acts on ADF when worms are starved so as to enhance the release of serotonin when food is again detected (Lemieux et al., 2015).

This circuit turns out to be even more complex, involving not just the monoamines but the neuropeptides pigment dispersing factor 1 and 2 (PDF-1 and PDF-2), which, like octopamine, promote roaming behavior. When either acts on the receptor PDFR-1 in RIM as well as RIA and RIB interneurons, it activates AVB neurons to promote forward movement, and hence roaming (Flavell et al., 2013). AVB neurons release PDF-2, creating a feedback loop sustaining forward movement. This circuit is countered by the serotonergic pathway in which NSM neurons inhibit neurons with a MOD-1 receptor (not just AIY, but also ASI and RIF neurons), which otherwise activate the PDFR expressing neurons. Drawing on these connections, Ji et al. (2021) advanced an account in which mutual inhibition between NSM serotonergic neurons and neurons with the MOD-1 serotonin receptor and the PDFR-1 receptor constitute a switch (Fig. 5). The positive feedback between AVB neurons and those with a PDFR-1 receptor serves to keep the switch in the condition for roaming. When AIA, a neuron that receives inputs from nearly all of the worm's chemosensory inputs, responds to food odors, it can act on NSM neurons to release serotonin and switch to dwelling. However, AIA also activates the neurons inhibited by NSM, thereby also promoting roaming. Ji et al. speculate that this “might allow both the roaming- and dwelling-active neurons to be latently activated when the animal is exposed to food odors detected by AIA. AIA-transmitted information about food odors could then be contextualized by other sensory cues that feed into this circuit.” One such sensory state would be the detection of food in the pharynx by NSM neurons, which then drives a more robust dwelling state than that achieved by just odors alone. Ji et al. noted that they could not identify just one neuron, comparable to NSM, regulating roaming but rather a number of neurons that are active

<sup>12</sup> One reason worms were initially only thought to sleep between stages of development is that in the laboratory when raised on a diet of *E. coli*, worms are perpetually starved. You et al. (2008) found that when satiated, worms cease foraging and feeding and become quiescent, adopting a sleep-like posture. The propensity of worms to enter the quiescent state depends in part on its registration of its internal metabolic state and in part on its previous history (a worm is more likely to enter quiescence if it had experienced periods of fasting). You et al. identified insulin (as detected by the *daf-2* receptor), TGF $\beta$  (*daf-7* receptor), and cGMP (*daf-11* and *egl-4* receptors) as promoting quiescence (all of which also figure in entry into the dauer state, discussed below). You et al. also made the observation that mutated worms that did not exhibit quiescence exhibited darker intestines, a sign of increased fat storage due to continued feeding. Gallagher et al. (2013) demonstrated that TGF $\beta$  is expressed in two ASI neurons and serves to block the transition from quiescence to dwelling and accelerates the transition from dwelling to quiescence. The ASI neurons inhibit RIC and RIM neurons that, as discussed above, release octopamine and tyramine respectively. These monoamines promote wakefulness. Makino et al. (2021) demonstrated that cGMP activity is further downstream and that the neuropeptide FLP-19 figures in initiating it in RIS neurons, which they present as further downstream of the RIC and RIM neurons.

<sup>13</sup> A further source of octopamine are bacteria in the guts of worms that produce and release tyramine, which the worms metabolize to octopamine (O'Donnell et al., 2020).

<sup>14</sup> Mutant worms unable to produce either octopamine or tyramine, generate both periods of roaming and dwelling, presumably reflecting the different effects of serotonin. Churgin et al. (2017) appeal to the dual effects of serotonin as well as the effect of octopamine to account of the fact that wildtype worms living on beds of *E. coli* spontaneously shift their behavior between roaming and dwelling: “Our results suggest that by continuing to release lower levels of the nondominant biogenic amine—octopamine under feeding conditions and 5-HT under fasting conditions—worms continue to maintain access to a variety of behaviors, some of which normally associated with an environmental state they do not currently find themselves in. By reducing, but not eliminating, behaviors associated with environmental conditions not currently being experienced, animals maintain a high degree of adaptability” (p. 7822).



**Fig. 4.** Roles of octopamine, released by RIC neurons and responded to by SER3/6 receptors (blue), and serotonin, released by ADF and NSM neurons responded to by several receptors (green), in regulating roaming versus dwelling.

at different times when the worm roams, leading them to suggest that roaming might be the default state of the network that is shut down by serotonin.

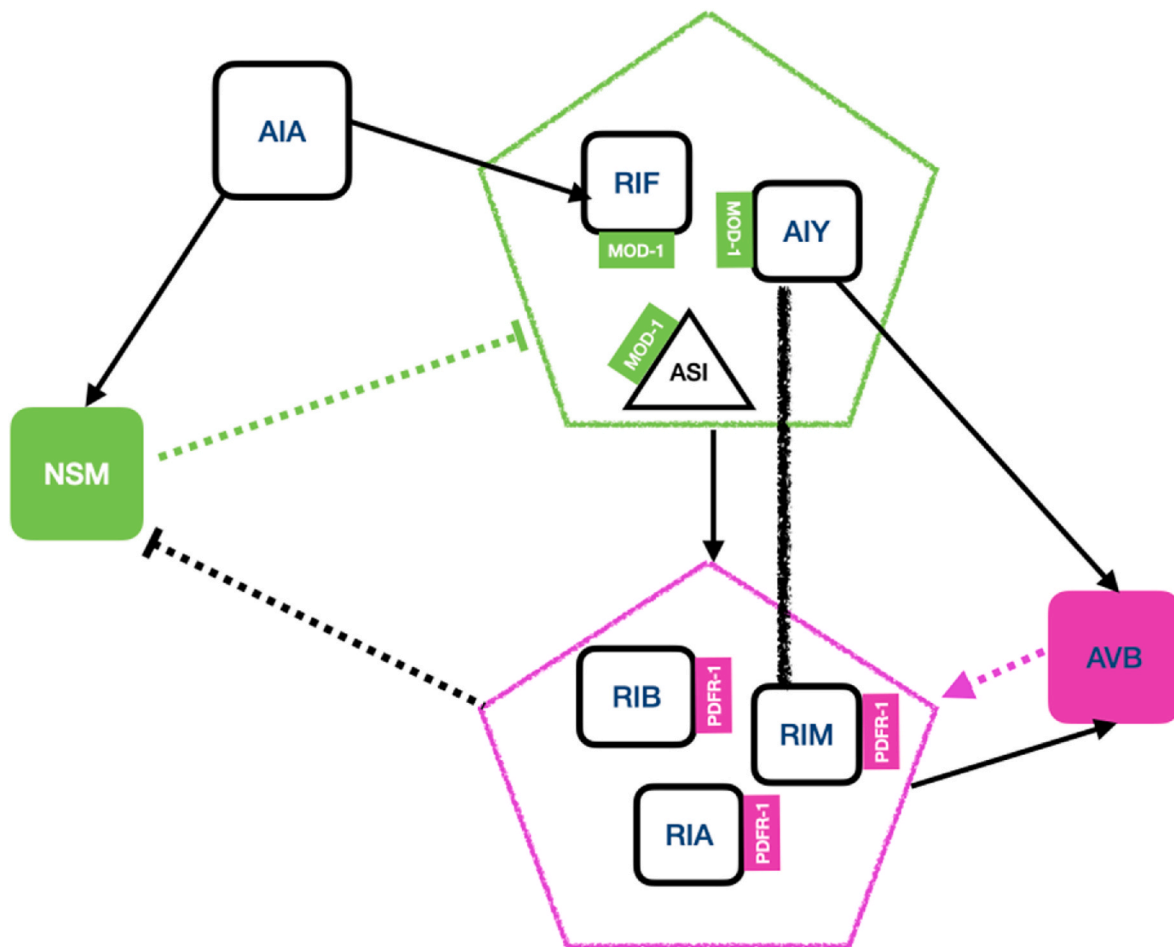
López-Cruz et al. (2019) investigated the time course of dwelling and roaming after feeding by removing worms from the site in which they were feeding. For the first 15 min the worms engaged in a local search strategy consisting of multiple turns, after which they adopt a global search strategy marked by fewer turns. The researchers construed the turns during local search as generated by an integrated network of RIM, AIB, and AVA neurons (shown, without interconnections, in the pentagon in Fig. 6). Normally the circuit and local search is promoted by AIA and ADE neurons, which in turn receive inputs from mechanosensory neurons such as FLP and chemosensory neurons such as ASK. (The chemosensory neurons also send direct inputs to the RIM, AIB, AVA circuit, not shown in Fig. 6.) In the absence of food, these sensory neurons send glutamatergic inputs to AIA and ADE neurons via MGL-1 GPCRs receptors. This is an example of an exceptional case, as noted in footnote 5, in which glutamate, which usually acts on ionotropic receptors, behaves as a neuromodulator acting on a metabotropic receptor, suppressing activity of AIA and ADE neurons for a period of 10–20 min. It thereby enables extensive turning movements, which is characteristic of local search. The activity of MGL-1 receptors decays over time, restoring the inhibition of turning, enabling exploration over greater distances. The researchers also view glutamate acting on MGL-1 receptors as accounting for worms ceasing pharyngeal pumping and, via transforming growth factor  $\beta$ , initiating metabolism of stored fat, as well as autophagy.

Although the presence or absence of food is the major factor governing feeding behavior, a variety of additional factors contribute to switching agendas and thereby alter worm feeding behavior. One is temperature. Mild increases in temperature results in increased serotonergic signaling and increased pharyngeal pumping, but high heat

reduces it. Subsequent release from heat stress also results in increased serotonin signaling and feeding (Ishita et al., 2020). The quality of food also matters. Worms are capable of detecting when they ingest noxious foods. Bhatla et al. (2015) describe how they first stop pumping and then resume at a faster rate. This has the effect of expelling the food currently in their pharynx.

Starvation not only causes worms to roam in search of food but to engage in activities such as sleep, as we discuss further in the next section. Perhaps the most dramatic response is when starvation occurs early in development, when it can have particularly serious consequences. Worms have developed a strategy, known as dauer diapause, for anticipating starvation that would impair their development and protecting themselves.<sup>15</sup> One indicator of potential starvation is abnormally high population density. Worms in the first larval stage (L1) can recognize this through their ASI sensory neurons, which detect increased ascariodes secreted by other worms (as well as extremely high temperatures or actual starvation). By inhibiting the release of the peptides that trigger normal molting and progression to L2, worms enter the predauer stage L2d. If the increase in ascariodes turns out to be a false alarm (recognized by the availability of  $\text{NAD}^+$  and other products generated as food is metabolized), worms proceed to molt and enter stage L3. But if they do not consume food, they enter the dauer state, undergoing large-scale changes of morphology, physiology, and behavior.

<sup>15</sup> In order to enter dauer worms require nutrients and if born totally deprived of food they do not enter dauer but rather arrest development (L1 arrest), a stage from which they can emerge if food becomes available within a couple weeks. In that case, worms proceed with normal development. Worms starved during L2 or L3 complete the molt for their current stage and arrest at the beginning of the next stage. In these cases, worms also resume development if food is subsequently available.



**Fig. 5.** Switch controlling states of dwelling and roaming. Release of serotonin from NSM acts on MOD-1 receptors (green) to suppress roaming. PDF releasing AVB neuron maintains activity in neurons with PDF receptors (magenta) that act to inhibit NSM. AIA activates both NSM and the neurons inhibited by NSM.

Morphologically, they manifest a thicker cuticle, a remodeled pharynx, and a narrowed body. Physiologically, they increase their stores of lipids and their metabolism changes. Behaviorally, they are mostly motionless, but they do react to vibration or touch and can even stand on their tails, wave their bodies, and attach themselves to insects or other animals. During this state they are highly tolerant to starvation as well as other stresses (Summers et al., 2015). They can remain in this state for up to four months (much longer than their usual one-month lifespan).<sup>16</sup> When an adequate food source is detected, worms exit the dauer state and live as normal adults.

Dauer can be viewed as an enduring state of the worm, comparable in some ways to roaming and dwelling. Instead of being established and maintained by monoamines, it is regulated by FMRFamide-like neuropeptides. Lee et al. (2017) identified over 8000 genes that are differentially expressed during dauer. Noting numerous neuropeptides that are upregulated during dauer entry, they developed mutants in which these were not upregulated and showed that these mutants did not enter dauer. They also established that the neuropeptides FLP-10 and FLP-17 figure in dauer-specific behavior including nictation behavior and switching from being repulsed by CO<sub>2</sub> to being attracted to it. Chai et al. (2022) found that most neuron classes express both peptides that promote and ones that inhibit dauer. They focused on the GPCRs that

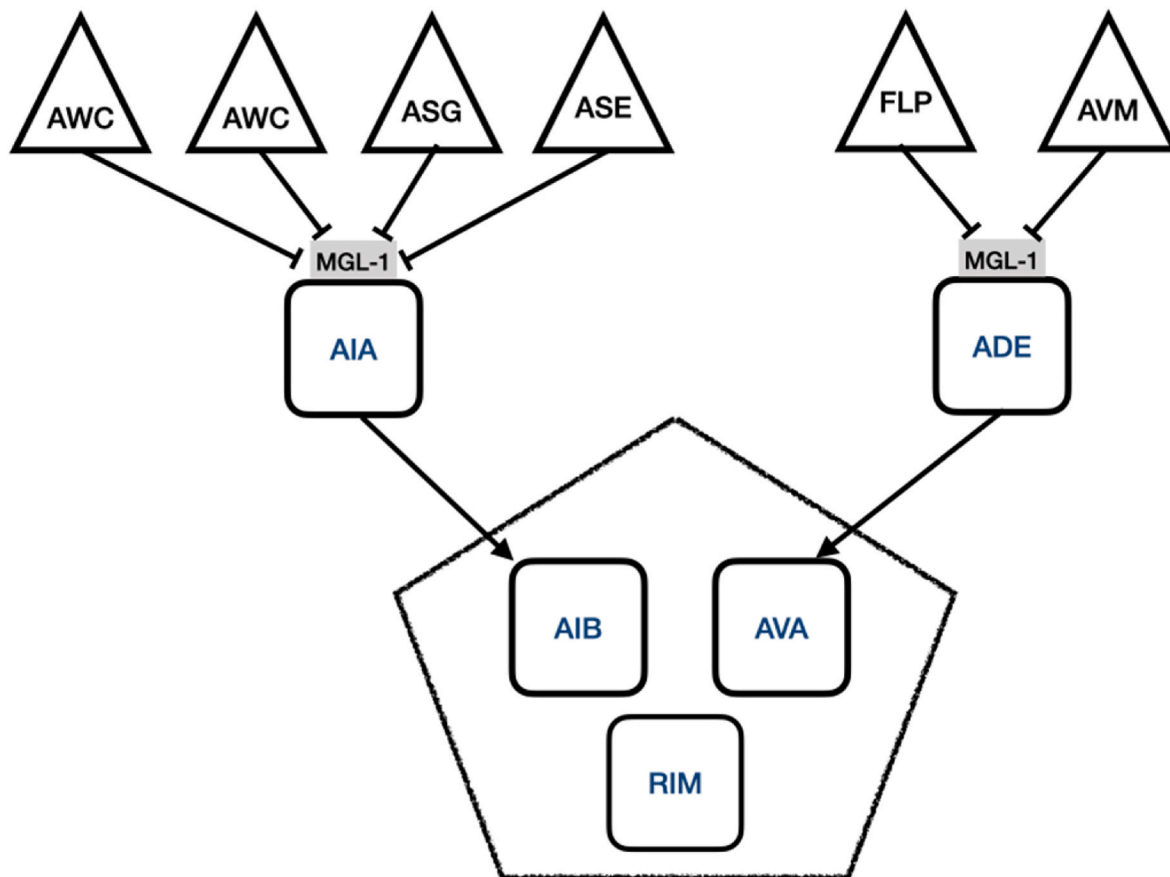
contribute to dauer, finding that all are expressed in neurons, with almost all classes of neurons expressing at least one. Four classes of neurons with the largest number of receptors, AVF, CAN, PVF, URX, are widely distributed through the organism. Chai et al. proposed that this provides “a means of coordinately regulating the whole-organism transition into the appropriate larval stage” (p. 4).

The decision to enter dauer is very consequential. Going through the dauer state results in changed behavior in the adult, including food seeking behavior—worms that have gone through dauer are more likely to dwell than to roam (Ackley, 2019; Pradhan et al., 2019). It also has effects on reproduction that persist into the following generation. Reversing dauer is an extended process. Worms require about 20 h to re-enter normal development. The transition into dauer is governed by a switch involving the nuclear receptor DAF-12, which then functions as a transcription factor for genes required for normal development. Dafachronic acids (DAs), which are generated in normal metabolism, act as a ligand for DAF-12. When DAs are absent, unbound DAF-12 receptors interact with the SHARP corepressor ortholog DIN-1S. This and other recently identified reaction pathways result in the altered development characteristic of dauer worms (for a detailed account of what is currently known about the initiation of dauer and changes in the dauer state, see Baugh and Hu, 2020).

In this section we have described some of the activities in which worms engage to procure the energy they require to maintain themselves far from equilibrium. The control of these various activities is complex, involving numerous neural circuits. These circuits involve not only classical synapses but also monoamines and neuropeptides that are released and diffuse through the worm to loci at which they bind

<sup>16</sup> The transcription factor DAF-16 is required for entering the dauer state, but a mutation in this gene can extend the normal life of the worm for up to an additional month, accompanied by increased stress resistance (Kenyon et al., 1993).





**Fig. 6.** The normal excitatory action of AIA and ADE interneurons on the integrated circuit consisting of AIB, AVA, and RIM is inhibited when AIA and ADE receive glutamatergic inputs to MGL-1 GPRCs, allowing the worm to engage in local search for food.

metabotropic receptors. These receptors operate on a slower timescale than ionotropic receptors, thereby serving to coordinate behavior over prolonged periods of time. Moreover, monoamine and neuropeptide circuits often work in coordinated opposition to one another in an arrangement that enables switching between different enduring states. A further feature of these circuits is that they enable the worm to coordinate responses to multiple sources of information that require different responses if the worm is to maintain itself. This feature is even more evident when we turn in the next section to how worms respond to various aversive stimuli.

#### 4. Adaptively responding to adverse environmental conditions

Lack of food is only one of many environmental conditions that threaten the ability of worms to maintain themselves. We turn now to threats and adverse conditions that cause worms to retreat. Worms have an integrated neural system that enables reversing forward movement and backing away when aversive stimuli are detected. After discussing the circuitry through which worms detect and retreat from aversive stimuli, we focus on how worms, based on different agendas, address situations in which they are both attracted to but also repulsed from a location such as when hunger directs a worm towards food but aversive stimuli direct it to retreat.

As shown in Fig. 7, two command interneurons, AVA and RMG, figure centrally in initiating reversal movements. AVA has gap junctions and chemical synapses to both backward (VA/DA) and forward (VB/DB) motor neurons but has many more to the backward motor neurons so that its overall effect is backwards movement. The polymodal ASH sensory neurons constitute one of the inputs to this circuit. ASH neurons extend a ciliated dendrite into the external environment that is capable

of sensing conditions such as high-osmolarity, high and low pH, the presence of blue or UV light, heavy metals, toxic volatile odorants, and detergents, as well as when objects touch its nose (for a detailed account, see Xu, 2015). ASH neurons have direct synaptic connection to the AVA command interneurons, but they also have an indirect connection through a circuit involving AIB and RIM interneurons. We noted above that among the transmitters produced by RIM is tyramine which has relatively long-lasting effects. This enables the worm to maintain a sustained response to an aversive stimulus. As demonstrated by Gordus et al. (2015), when the same circuit of interneurons receives inputs from the odor-sensing AWC neurons, RIM generates complex dynamics within the circuit that induces delays in the response when the odors diminish. Depending on the state of the RIM and AIB interneurons, the circuit will result in different responses. Moreover the response is probabilistic (see Kristan, 2015, for a discussion of the importance of such probabilistic neuronal circuits in explaining overall organism behavior).

A variety of odors cause worms to withdraw.<sup>17</sup> For example, when presented with an eyelash or pipette tip dipped in 1-octanal, an ester with a pungent odor, worms back off, doing so faster in response to higher concentrations (in 3–5 s with 100% octanal, 8–10 s with 30%). Investigators have built upon this protocol to explore how the response varies with other conditions such as food availability. When exposure is coupled with octopamine, signaling starvation, a worm withdraws fully,

<sup>17</sup> Much of the research on odors focuses on aversive ones. But worms also respond positively to some odors. Dobosiewicz et al. (2019) have demonstrated that AIA neurons respond to attractive odors, suggesting that its activation represents the assignment of positive valence to an odor. AIA neurons are activated by AWA olfactory neurons together with a suppression of activity in neurons responding to aversive stimuli.

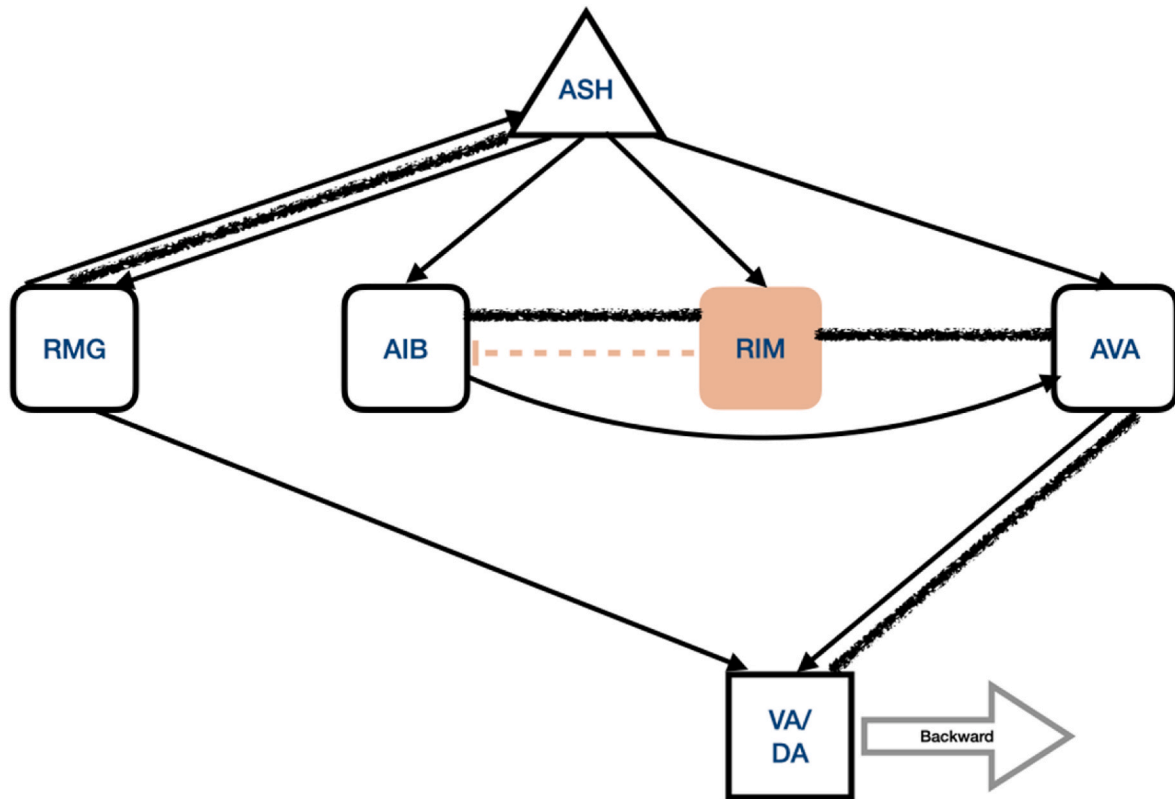


Fig. 7. Simplified version of the circuit from ASH sensory neuron to VA/DA neurons directing backwards movement.

swinging its head two or more times, and makes a substantial turn ( $>45^\circ$ ). But when the RIM neuron releases tyramine, signaling the presence of food, the worm only briefly backs off and then continues its grazing movement (Komuniecki et al., 2012). A likely mechanism for this altered response involves ASH directly acting on AVA to initiate withdraw followed by tyramine from the RIM neuron inhibiting AIB and preventing it from maintaining the signal to withdraw (Summers et al., 2015).

Ghosh et al. (2016) investigated how worms decide between locomotor options by positioning worms within a hyperosmotic ring that could desiccate them. Only 30% of well-fed worms crossed the barrier. However, if an attractive scent, indicating food availability, originated from outside the ring, 80% crossed the barrier. If the worms had been food deprived for 1 or 5 h, the percent crossing the barrier was even greater. The researchers identified and then modeled a small network consisting of a pair of sensory AWA interneurons activated by the attractive odor, the pair of polymodal sensory ASH neurons that detected hyperosmolarity, and a pair of sensorimotor RIM interneurons (Fig. 8). The RIM neurons receive excitatory inputs from the ASH neurons and inhibitory inputs from the AWA neurons and send inhibitory gap junction connections to interneurons that command forward movement and excitatory connections to interneurons that command backward movement. The result is that when ASH neurons alone are active, signaling hyperosmolarity, they promote withdraw, both directly and via RIM neurons. When AWA neurons are active, they inhibit RIM. This allows forward movement. RIM neurons also express the neuropeptide PDF-2 and the GPCR PDFR-1, resulting in a positive feedback loop that serves to maintain RIM activity. In mutants lacking PDFR-1 this sustaining feedback does not occur, rendering worms much more likely to cross the ring. RIM neurons, as discussed earlier, also express tyramine for which the ASH neurons have receptors, resulting in another positive feedback loop. Mutants without tyramine do not exhibit enhanced ASH activity and are more likely to cross the barrier than wildtype worms. As we discussed above, tyramine synthesis decreases with hunger,

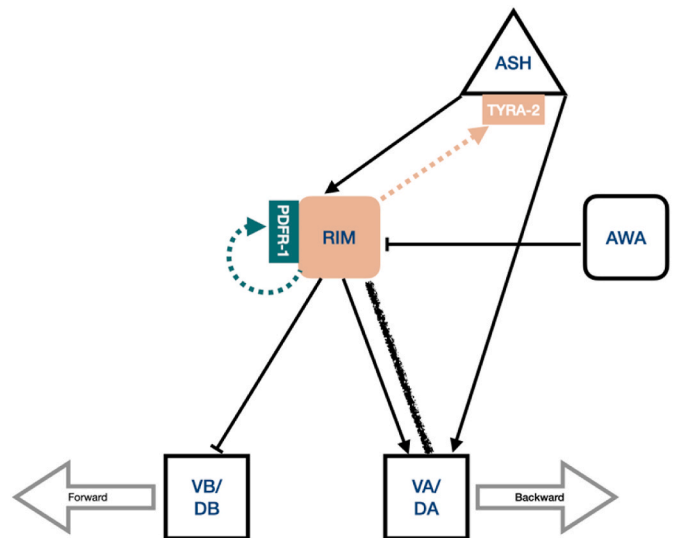


Fig. 8. Circuit promoting withdrawal from noxious stimulus. RIM feeds back positively on itself via expression of the neuropeptide PDF-2 and the receptor PDF-1. It also produced tyramine, through which it feeds back on ASH via the TYRA-2 receptor.

rendering hungry worms, like the tyramine mutants, more likely to cross the barrier and well-fed worms less likely.

Subsequent research has revealed additional neurons that interact with this circuit, many employing monoamines, enabling worms to integrate these responses with those to additional signals. For example, ASI are another pair of polysensory neurons that respond to food odors and have been shown to affect many activities discussed above: entrance into the dauer state, satiety quiescence, exploratory locomotion. Guo

et al. (2015) showed that ASI neurons inhibit ASH neurons in a manner that is dependent on the ASI neuron's response to serotonin via the SER-5 receptor (Fig. 9). Guo et al. proposed ADF neurons are intermediates in the action of ASI on ASH. ADF neurons are serotonergic sensory neurons that act on the SER-5 receptor on ASH, inhibiting it.  $\text{Cu}^{2+}$  is an aversive stimulus that acts on all three sensory neurons, but with different effects. Its action on ASH is rapid and robust. ASH neurons excite octopamine-synthesizing RIC neurons. The octopamine they release acts on the SER-3 receptor on ASI neurons, inhibiting them.  $\text{Cu}^{2+}$  acting on ASI neurons gates the action of  $\text{Cu}^{2+}$  on ADF (probably through neuropeptide signaling), resulting in release of serotonin that inhibits ASH. Guo et al. interpret this interaction as allowing for the finetuning of the worm's response to  $\text{Cu}^{2+}$ .

In this example the ASI and ASH neurons provide competing responses to the same stimulant, but they also respond to different stimuli. Whereas ASH neurons detect numerous aversive stimuli, ASI neurons respond to nutrients.<sup>18</sup> Davis et al. (2018) focused on how the two neurons affect behavior in starved versus well-fed worms. They found that in starved worms, the response of ASH neurons to aversive stimuli was decreased while that of ASI neurons to nutritious stimuli was increased compared to well-fed worms. They also examined the effects of lesioning either pair of neurons on satiety quiescence, finding that without ASI neurons, worms fail to switch to quiescent behavior, while without ASH neurons, worms spend more time in the quiescent state. They concluded that the nutritional state determined the worm's response to aversive stimuli by altering the respective influence of ASI and ASH neurons.

To illustrate the systems by which worms respond to aversive stimuli and integrate these responses with those to attractive stimuli such as food, we have focused on responses to only one or two sensory neurons. These neurons can themselves generate different responses to different stimuli, and as Ghosh et al. (2017) describe, sensory neurons modulate each other's behavior and interneurons integrate responses from multiple sensory neurons. As Ghost et al. emphasize, "Multisensory integration, especially when combined with a survey of the internal physiological state, provides selective advantages to animals navigating complex environments."

Some bacteria are pathogenic to worms. For example, *Pseudomonas aeruginosa* disrupts the intestinal lumen, *Bacillus thuringiensis* generates a crystal pore-forming toxin that disrupts ingestion, and *Microbacterium nematophilum* adheres to the rectal and post-anal cuticle. When harmful bacteria are detected in the pharynx, worms can halt normal grinding and expel the current contents. When pathogens reach the gut and infect the worm, it often initiates an innate immune response (Singh and Aballay, 2019). Worms also learn to avoid those bacteria which have made them ill (Kim and Flavell, 2020). We will focus on a response to illness that is common among animals but only recently discovered in worms—sleep. As we noted above, sleep in worms was initially only identified in transitions between larval states, not in adult worms. Researchers have more recently demonstrated sleep behavior after exposures to pathogens that result in sickness (Hill et al., 2014; DeBardeleben et al., 2017)<sup>19</sup> and during prolonged starvation (Skora et al., 2018; Wu et al., 2018). Davis and Raizen (2017) argue that sleep during illness redirects energy from motor tasks to address the illness or injury. Typically this sleep state is maintained for a period after the stress is removed, suggesting that sleep may contribute to the recovery from

<sup>18</sup> ASI neurons are also responsive to the endogenous opioid NLP-24, which is released during starvation (Rengarajan et al., 2019). show that hunger can reverse the response of worms to some sensory cues: CO<sub>2</sub> repels well-fed worms but attracts starved ones, with dopamine playing a major role in avoiding CO<sub>2</sub> and octopamine generating attraction, relying on an antagonism between AIY and RIG.

<sup>19</sup> Sleep has also been found in response to bacterial toxins, heat shock, cold shock, osmotic shock, and ultraviolet light exposure.

stress (e.g., through repair of misfolded proteins) (Fry et al., 2016).<sup>20</sup> Moreover, the sleep period is extended proportionate to the degree or duration of the stressor.

Like lethargus sleep, stress-induced sleep employs serotonergic RIS to inhibit waking activities. However, the circuit that elicits RIS activity is different than in lethargus (Trojanowski et al., 2015). A key role is played by the ALA interneuron (Fig. 10), which appears to play little role in lethargus.<sup>21</sup> In addition to activating RIS, ALA itself acts to suppress waking activities through release of the neuropeptides FLP-13, FLP-24, and NLP-8. Trojanowski et al. (2015) present evidence that the way in which this inhibition acts to stop pharyngeal pumping is different than in lethargus sleep—in lethargus sleep it acts on the muscle itself whereas in stress-induced sleep it acts on motor neurons. Activation of ALA is brought about by epidermal growth factor (EGF) acting on the ALA neuron, depolarizing it (Flavell et al., 2020).

To maintain themselves, worms need to respond appropriately to a host of adverse conditions that occur in their environment. In each case, sensors that detect the condition need to be linked to motor activities through which worms can take appropriate action. But this is not sufficient. In some cases, worms will fare better if they continue activities such as seeking food even when conditions are adverse. They need to detect a variety of conditions, including ones within them, and integrate their responses to them. The examples we have provided illustrate some of the ways worms coordinate their response to adverse stimuli with other demands.

## 5. Conclusion: maintaining autonomy with neurons

Theorists that constitute the biological autonomy tradition, from pioneers such as Piaget (1967), Rosen (1972), and Maturana and Varela (1980) to more recent contributors such as Kauffman (2000), Moreno and Mossio (2015) and others, have characterized biological organisms as organized systems that are far from equilibrium and that maintain themselves through their own actions. These theorists have appealed to some variation of the notion of *organizational closure* to explain how organisms perform the activities they need to perform to maintain themselves. This notion characterizes the organization common to all living organisms as a circular network of components and production processes in which, for each component C1, the conditions necessary for its production and maintenance are determined by another component C2, whose existence and maintenance depends, directly or indirectly (through other components), in turn, on C1. Different theorists have advanced different accounts of what, specifically, is closed: Maturana and Varela (1980) invoke closure of processes, Rosen (1991) closure of efficient causation, and Moreno and Mossio (2015) closure of constraints. All of them, however, characterized these abstractly and have not elaborated on how organisms actually maintain themselves. Bich and Bechtel (2022b) have argued that to explain how organisms maintain themselves as autonomous systems, it is not sufficient to focus only on closure of productive components. Closure needs to be complemented with an account of how the activities of components are

<sup>20</sup> Goetting et al. (2020) demonstrated that it was actual damage, found with disruption of proteostasis or DNA repair, not the perception of noxious conditions, that trigger SIS sleep. When protein homeostasis was restored, the worms did not extend their sleep but when DNA repair was impaired, sleep drive was increased. They also demonstrated increased SIS following physical injury, especially to the midbody of the worm. Pursuing the question of whether energy deficiency, registered by phosphorylation of AMPK, triggered sleep, the researchers found the opposite—energy deficiency results in reduced stress-induced sleep, and inferred that the worms put off bodily repair until they had sufficient energy.

<sup>21</sup> Lethargus instead requires the paired RIA neurons, which release the neuropeptide NLP-22, and the RIS neuron, which requires the transcription factor APTF-1 (Nelson et al., 2013; Turek et al., 2013). Neither NLP-22 nor APTF-1 is required for stress-induced sleep.

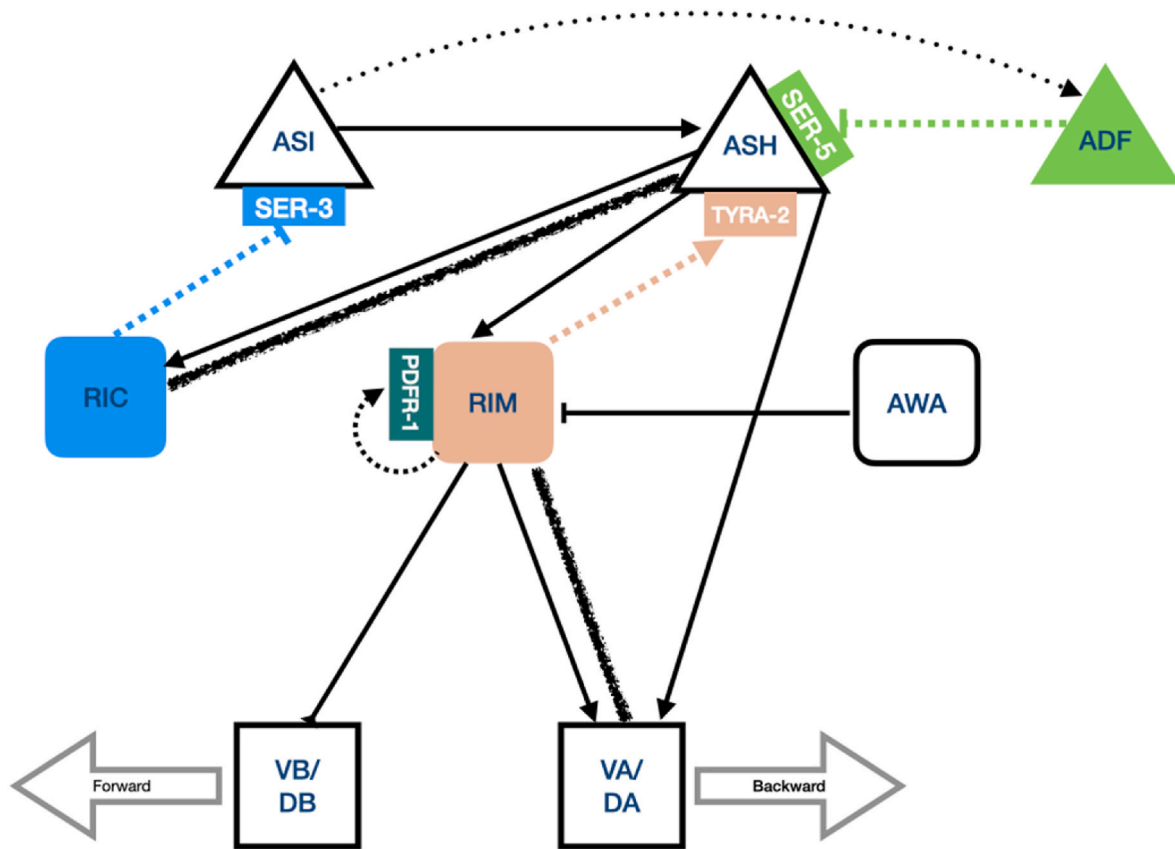


Fig. 9. Two neurons interact with ASH, modifying its response. ADF releases serotonin that acts on the SER-5 receptor on ASH. ASH activates RIC neurons that express octopamine, which acts to inhibit ASI's input to ASH.

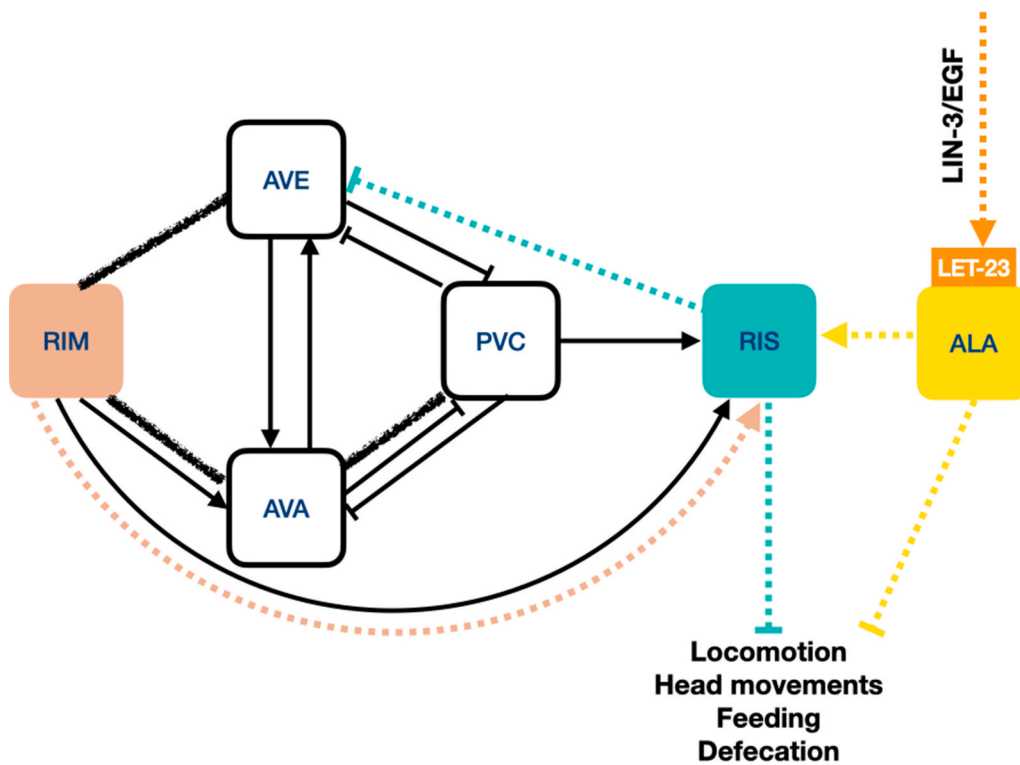


Fig. 10. Extension of Fig. 2 to illustrate how, in the case of stress-induced sleep, ALA complements RIS in shutting down activities that are executed while awake. Many details are not known, but ALA is known to respond to epidermal growth factor LIN-3 (orange), which acts on ALA through the LET-22 receptor and that it acts via the neuropeptides FLP-7, FLP-13, and FLP-24 (yellow).

controlled from within the system. As a contribution to providing a substantive account of how multicellular organisms control the basic mechanisms through which they maintain themselves, we have examined some of the processes employed in the worm, both those involved in procuring food and avoiding adverse conditions. In doing so, we have illustrated how ideas from the autonomy tradition about self-maintenance and control can be grounded in actual biology. The worm provides a useful model for understanding the basic processes of neural control since, lacking a vascular system, it employs its nervous system to coordinate the diverse cells that constitute it.

Even with relatively few neurons and without a centralized brain, the worm is able to control a host of behaviors so that they are performed when they are appropriate given the state of the worm and the conditions it confronts. As we have illustrated, the control processes regulating each activity are local to it and involve circuits with relatively few neurons. They nonetheless enable the worm to integrate diverse sources of information and perform its activities when and as needed. This is a feature that is conserved even in organisms with a centralized brain—the basic control mechanisms are achieved by neurons in different nuclei that are connected to the productive processes they regulate. In mammals, the control of activities such as feeding, avoiding aversive conditions, and sleep are regulated by neurons in nuclei in the brain stem such as the nucleus of the solitary tract. Neurons in higher centers such as the hypothalamus integrate the different activities, but control remains distributed between different nuclei that carry out specific integration. One might think that without a central executive overseeing all activities, organisms would not be able to effectively coordinate the activities they must perform. The worm, however, shows that this is not necessary.

The nervous system is often conceptualized as an electrical signaling system. The transmission of current along neurons fits that conception of the nervous system. But our examination of the worm illustrates that it is considerably more complex. In particular, in addition to transmitters such as glutamate and GABA acting on ionotropic receptors, worms make extensive use of monoamines and neuropeptides acting on metabotropic receptors. These importantly enable the worm to maintain agendas, set by their assessment of their internal state, over relatively long periods. They enable the worm to process information in different ways depending on conditions within the worm and its environment so as to carry out concerted activity. Such neuromodulators play an important role in mammals such as us as well, and the worm provides a useful model in which to appreciate their significance.

Biologists commonly refer to the systems that perform the different activities in organisms as mechanisms, and recent work in philosophy of science has attempted to explicate how mechanisms figure in biological explanations (Machamer et al., 2000; Bechtel and Abrahamsen, 2005). Some of the mechanisms biologists identify directly perform the activities organisms must carry out to live such as contracting muscles. These can be characterized as production mechanisms. But in order for production mechanisms to operate when and in the manner needed, organisms must control these mechanisms with other mechanisms, control mechanisms (Winning and Bechtel, 2018; Bich and Bechtel, 2022b). Control mechanisms act on production mechanisms (as well as other control mechanisms), altering their activities in response to measurements they carry out of relevant conditions. Recent work on unicellular organisms has identified four main features of control: (1) measuring appropriate variables and acting upon those measures in a manner that (2) is dynamically decoupled from basic metabolism, while (3) allowing for intermediate components to integrate different measurements and effects, and yet (4) enabling sufficient segregation to achieve specific responses (Bich and Bechtel, 2022a). In multicellular systems control exhibits similar features but involves intercellular as well as intracellular activities. The neural systems we have described in the worm are such multicellular control mechanisms. Neurons are sensitive to features of the internal and external environment of the organism and operate accordingly. They do so in a way that does not directly depend on their

internal metabolic state but on what they sense. Intermediate steps can involve the action of further neurons (e.g., interneurons), thus integrating their measurements. And segregation can be achieved through physical boundaries, the organization of the synaptic network and, such as in the case of neuromodulators, by means of expressing different membrane receptors.

Often control, both in social institutions and in the nervous system, is envisaged as hierarchical, with a top-level controller overseeing lower-level controllers. The worm shows that this is not necessary. There is no centralized control in the worm, but a distributed network of neurons organized into local circuits both regulating specific activities and integrating their activities with each other. Moreover, studying neural control in the worm brings to light a further general feature of biological control. Control mechanisms not only transiently modulate other mechanisms at specific points in time, but also set agendas that affect those mechanisms over sustained periods of time. This means establishing, maintaining and shifting between different global behavioral and physiological regimes such as feeding, dwelling, sleeping and dauer, which endure for a prolonged time depending on the state and needs of the organism. By examining some of the neural circuits in the worm, electrical and chemical, we have attempted to move beyond the abstract conception of biological autonomy and focus on the importance of control by providing a more detailed understanding of how neurons enable organisms to maintain themselves. We have, however, only discussed how worms regulate eating and responding to threats. There are many additional activities that they regulate that bear further examination. Moreover, we have emphasized that distributed control processes suffice to coordinate behavior, but just how distributed control facilitates coordination also merits further investigation. Finally, we have restricted ourselves to the activities in the nervous system. A future step would be to analyze the connection between the motor neurons and those physiological and metabolic activities involved in self-production of the worm's components.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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