Peer-reviewed version available at *J. Dev. Biol.* **2019**, *7*, 8; doi:10.3390/jdb7020008

Functional connectomics in *C. elegans*

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Peer-reviewed version available at J. Dev. Biol. 2019, 7, 8; doi:10.3390/jdb7020008

Abstract

The complete structure and connectivity of the *Caenorhabditis elegans* nervous system was first published in 1986. The 'mind of a worm' was the first organism to have its nervous system to be reconstructed at the level of synapses, and represented a critical milestone considering today it remains the only organism to be mapped to that level of connection. Recently, the extrasynaptic connectome of neuropeptides and monoamines has been described. This review discusses recent technological advances used to perturb whole-organism neuronal function, such as: whole brain imaging, optogenetics, sonogenetics and mutant analysis, which have allowed for interrogations of both local and global neural circuits, leading to different behaviors. A better understanding of a whole organism requires combining experimental datasets with biophysical neuronal modelling, and behavioral quantification. Combining these approaches will provide a complete understanding of the worm nervous system and shed light into how networks function and interact with the synaptic network to modulate information processing and behavioral output.

Peer-reviewed version available at J. Dev. Biol. 2019, 7, 8; doi:10.3390/jdb7020008

Introduction

Extensive research on the *Caenorhabditis elegans* neural connectome dates back to 1986, leaving them rooted in the minds of neuroscientists ever since. Investigation into the physical connections of the neural pathways by electron microscopy predates the naming of this research field (1). Knowledge of the brain's structure has improved exponentially over the past century; before which, focus was on gross anatomy and discrete brain regions. The shift to how neurons connected at the beginning of the 20th century with debates between Camillo Golgi and Santiago Ramón y Cajal over the connections between neurons (2). Initially referred to as "collation of connectivity data," the term "connectomics" was popularized in 2005 with regards to the human connectome (3, 4). This field was brought to public consciousness by 2012, alongside the "Human Connectome Project" and reinforced the following year with the Brain Research through Advancing Innovative Neurotechnologies® (BRAIN) Initiative (5-10). One overarching goal in neuroscience is to understand what structural components of a neural circuit relate to certain behaviors. Understanding this relation is not possible with the connectome alone, though it provides a strong foundation. One neuron could be involved in one behavior, but one behavior could be tied to a circuit of neurons. However, all behaviors result from summation of actions that take place within the connectome.

Caenorhabditis elegans

The importance of the connectome comes from the understanding that structure can influence function. Knowing the structure of a network is critical for understanding the function. Historically, most of the brain's functions have only been understood when there was injury to isolated structures; as was the case with Phineas Gage and others whose

injuries advanced scientific knowledge (11). Due to ethical considerations, there is a reliance on retrospective studies, dependent on injuries, to understand function. The appeal of model organisms becomes apparent given the cost of care, generally shorter life span, and simpler structure. *C. elegans* are a microscopic worm that has modeled macroscopic research for six decades (Figure 1). The nematode's wiring circuit, identified by electron microscopy in 1986, was one of the original renderings of a connectome for any organism (1). Over three decades later, it remains the only organism with a completely mapped connectome.

With model organisms the focus therefore becomes microscopic – in scale, not significance because it is possible to generate diseased or genetic homologs. One technique used to understand the shape of connections is microsurgery, which takes place at the level of individual neurons. Immobilizing a *C. elegan*, it is possible to use a laser to ablate neural bodies (12, 13). Laser ablations are possible in *C. elegans* because all of the neurons have been mapped and they are eutelic, making it possible to ablate the same neuron across multiple animals (14). This technique has power in manipulating structure. Worms can later be observed to see how behavior and function are related to structure. These types of studies are important, as damage of physical destruction to connections allows for the consideration as to how structural changes impact function and signaling between neurons. These changes to neuronal structure can be achieved across generations by using genetic ablations by expressing varying toxins or caspases (15).

One of the most commonly used tools in identifying structures in human models is Magnetic Resonance Imaging, or MRI. First image generated in 1973, this technique

utilizes a magnetic field created within the MRI machine to align the protons of water molecules within the sample (16). This information can be analyzed to provide structural data of the sample. Variants of this technique include diffusion MRI, which is of particular interest when studying the brain, as it can differentiate white matter tracts from gray matter. This technique isn't used to understand the structure of individual neurons or small scale circuits, but rather of the whole brain. It has tremendous strength in identifying abnormalities in structure and large pathways between regions (17).

Non-human models make use of many other techniques in determining the structure of the nervous system, however. While MRI relied on the location of protons, electron microscopy measures electron density. This method requires a fixed sample to be cut nanometers thick before staining and photographing the sections. Software then compiles these images into a 3D reconstruction of the image. In the case of *C. elegans*, a worm less than 1 mm in length from tip to tail, the initial process of imaging rendered 8000 images that had to be compiled (1). It is not difficult to imagine why this technique may take some time to analyze a larger, more complex organism, like a human as the BRAIN Initiative hopes to achieve (7).

Using *C. elegans* grants a better understanding of the mechanisms and possible treatments for diseases. In neuroscience especially, the central goal is to understand how the nervous system functions under typical and atypical circumstances (18). However, despite knowing the physical connections of the *C. elegans* nervous system, much remains to be learned about how the nervous system functions as a whole to enact behaviors (19). This involves understanding neuronal dynamics that extend beyond anatomical synapses, such as how neuromodulators can act over longer distances and

timescales to shape behavioral responses. Further complexing our understanding of this "functional connectome," is how the same neuron is capable of playing different roles in two different circuits.

Functional Focus

C. elegans neurons have been grouped into three functional "classes" of neurons: sensory neurons, motor neurons, and interneurons. The sensory neurons account for a third of neurons, and have more connections pre-synaptic than post-synaptic. Contrarily, motor neurons, another third of all neurons, have more post-synaptic connections. The remaining neurons are considered to be interneurons, with large numbers of both pre- and post-synaptic connections (20). These structural components can be understood in the context which they aid function. With more pre-synaptic neurons, sensory neural bodies are able to gather more signals from surrounding neurons. Post-synaptic connections within motor neurons allows them more synaptic connections with muscles to therefore produce fine-tuned actions. Understanding the connections alone between these neuron classes helps increase our understanding of how a signal is transduced, processed, to ultimately produce actions, as seen in previous work on *C. elegans*' navigation (21). However, these actions cannot be understood by the static connections alone.

It is well documented how in the *C. elegans* connectome, a single neuronal class can be involved in the sensation of diverse stimuli or elicit different behaviors. For example, the polymodal, nociceptive neuron, ASH, detects a myriad of different mechano-, osmo-, and chemo- stimuli that result in aversive behavior (22-28). However, not all

stimuli utilize the same pathways and connections as one might expect given detection by a single sensory neuron serving as progenitor for these circuits.

The diversity in neuronal circuitries may be due to the intracellular machinery used within individual neurons. *C. elegans* is equipped with a large set of G protein subunits that exhibit overlapping expression, rendering particular intracellular pathways important in different behavioral circuits (29). The nematodes genome codes for 21 Gα protein subunits, and 2 subunits of both Gβ and Gγ proteins (29). Of the 21 Gα subunits, 16 are expressed throughout the chemosensory neurons, and many overlap in their expression profiles (29). For example, on its own, ASH expresses ten different Gα subunits, while ASE expresses only three (29).

One of the earliest methods to monitor neuron function was patch clamp electrophysiology, a technique first used on *ex vivo* giant squid axons (30). This technique lends itself to understanding functional connectomics as it monitors the flow of ions across neuronal membranes (31). In *C. elegans* it was used to understand the role of graded potentials, in opposition to mammals all or nothing action potentials (19) (31). This method is still rather invasive, requiring fixed samples of individual neurons for testing (32). There are however, emerging techniques that allow for measurements of single cells within a larger neuronal structure.

It is possible to elucidate the functional connectome in less invasive ways than when determining the structural connectome. However, this often means that the images focus on individual neurons but the sample would not be permanently modified by the imaging procedures. A variant of MRI, functional MRI (fMRI), connects structure and function within *in vivo* samples (33). In these scans, a subject can be asked to perform a

task or to think of a topic, and the blood flow within their brain can be tracked (34). This set up can also be manipulated to expose a sample to a particular stimuli and the responses monitored. In this way, it is even possible to use a non-human model.

Techniques used pan-neuronally effect all neurons in a region surrounding the area of interest with the use of chemicals or induced electrical stimulation. Manipulation of a single neuron, such as in laser ablation, permanently alter structure before testing for a change in function. Optogenetics offers temporal control of activity of individual neurons utilizing light-controlled ion channels. This modification requires the insertion of opsin genes, responsible for proteins such as channelrhodopsin-2, natively found in green algae or *Chlamydomonas reinhardtii* (35). The activity of a single neuron can be controlled by this method. Similarly, calcium imaging allows for the activity of a single neuron to be monitored (36). As in optogenetic studies, genetic manipulation is involved, but in this case using fluorescent tags such as GFP or mCherry fused to the calcium binding protein, calmodulin, resulting in Genetically Encoded Calcium Indicators (GECIs). These GECIs are ideally localized to the neural body and or whole neuron. The technique is possible as it is known which genes localize to different neuron types and locations. In this technique, *C. elegans* is immobilized in a microfluidic device, and calcium levels are measured via changes in fluorescence of the GECI (37, 38).

Divergent functions within a neuronal class

The sensation of diverse stimuli as understood via the connectome is also seen in work on multisensory integration. While multisensory integration work has been conducted with humans using techniques such as magnetoencephalography (MEG) and fMRI, and also with model organisms such as *Drosophila melanogaster*, the results are

limited (39-41). Human studies of multisensory integration typically have extremely small sample sizes, with one recent study having data for a total of 26 individuals (39). Other than *C. elegans,* no model systems has a fully mapped connectome and with approximately 100,000 neurons in *Drosophila*, compared to the 302 in *C. elegans*, science still needs time to finish its next connectome and will also have to overcome the obstacle of much more complex neural circuitry (13, 42, 43). For example recent work on the multilevel multimodal convergence circuit for rolling in *Drosophila*, which relies on multisensory integration, was limited by the lack of knowledge surrounding multisensory neuronal convergence (44). All of these factors, especially the comparatively well understood *C. elegans* connectome, combined with what is otherwise often cost-prohibitive methodology, make *C. elegans* an ideal choice for multisensory work.

Multisensory methodology utilizing *C. elegans* falls into two broad categories; co-exposure to two stimuli, one that is aversive and one that is agreeable, or exposure to one stimuli in conjunction with an environmental indicator, both of which can be done via avoidance assays (45). The avoidance assay, also commonly known as the drop test was pioneered by in the early 2000s, and has transformed how researchers can approach multisensory integration research in *C. elegans* (46). The stimuli is dropped on the tail of the animal and through capillary action, this droplet quickly reaches the anterior amphid sensory organs, ultimately resulting in the *C. elegan* either continuing movement in the same direction or moving backwards to avoid the stimuli (46). Both methods of multisensory exposure within the scope of avoidance assays are comparatively simple and inexpensive, which also allows for more robust data. Furthermore, with only 60 ciliated sensory neurons, including 16 pairs of chemosensory neurons neuronal ablation

techniques such as the laser ablation method previously discussed as well as genetic ablation become effective tools for understanding the diverse roles of individual sensory neurons with respect to the connectome as a whole (47, 48).

Examining one neuron in particular, ASH, studies have revealed differential use of both intra- and inter-signaling molecules by the neuron upon detection of various stimuli, yet still resulting in the same behavioral outcome: avoidance (24, 27, 28, 49-51). For example, nose touch avoidance, which is assayed by allowing the animal to run into an eyelash positioned perpendicularly to the animal's movement, requires expression of *itr-1* in ASH (Figure 2A) (49). Yet *itr-1* have not been found to be necessary for osmotic aversive responses mediated the same neuron (Figure 2A) (49). This implies that specific G protein subunits are utilized by the same neuron in response to individual stimuli, in turn activating unique pathways within the neuron and ultimately deciding which synapses relay the response, establishing the functional connections. Indeed, response to nose touch and benzaldehyde require *itr-1* in ASH neurons (49). However, only nose touch requires IP3 production via phospholipase C enzymes *egl-8* and *plc-3* (49). It is therefore likely that the upstream Gαq subunit, *egl-30*, is only involved in nose touch, but not benzaldehyde detection (49).

Downstream of the initial sensation of stimuli, differences in functional post-synaptic connections have also been observed. As with *itr-1,* the glutamate receptor, *glr-1,* is utilized primarily in nose touch avoidance, as well as regulating subtle reactions within of osmosensation (Figure 2A) (28, 50, 51). Conversely, specific genes within ASH, such as *osm-10,* are specific to osmotic detection, and not tactile response. These genes have been shown to have specific downstream targets, such as *nmr-1* (Figure 2A) (24,

27, 51). Thus, it is possible different stimuli evoke different intra-signaling pathways which lead to varied synaptic release profiles to enact specific downstream targets in a functional circuit. Supporting this notion is the presence of both clear synaptic and dense core vesicles in ASH (1). Furthermore, it has been shown in ASI neurons, that depending on the signaling molecule, different neuropeptides are released from distinct neuronal compartments and asymmetrically between the neurons in the pair (52).

While certain intracellular components and synaptic connections are vital in some behaviors, they may be irrelevant in other behavioral circuits which utilize the same neurons. One example of this is the amphid sensory neuron, ADL, and its involvement in the response to ascaroside #3 (ascr#3). Ascarosides, are small-molecule signals, which serve diverse functions in inter-organismal chemical signaling (53). Hermaphrodites are observed to avoid ascr#3 through ADL chemical synaptic transmission, presumably, to the backward command interneurons AVA and AVD (1, 54). Promotion of ADL response to ascr#3 is achieved through the gap junction hub-and-spoke RMG circuit, wherein the interneuron RMG serves as a hub to modulate sensory neuron responses (54, 55). RMG, through the activity level of the neuropeptide receptor *npr-1*, and input from the sensory neuron ASK, can inhibit ADL triggered avoidance by altering gap junction properties (54, 55). Thus, chemical synapses are involved in the avoidance to ascr#3, whereas gap junctions are necessary for modulating the response in an *npr-1* dependent manner to elicit aggregation or attraction (Figure 2B).

Sex Differences

As an androdioecious species, *C. elegans* are primarily hermaphroditic, with only 0.1% of the population being males (56, 57). As such, most connectome efforts and

studies have identified 302 neurons in hermaphrodite (13). Despite the male connectome not being completely mapped, much has been studied in regards to the sex-specific differences in the 385 neuronal cells present in the male (58). The increase in neurons has been shown to be localized in the tail and head of the animal, and largely function in finding and reproducing with a mate (58-60). Furthermore, sex-specific circuits have been identified that govern the male response to sex pheromones, demonstrating the importance of fully mapping neural circuits in both hermaphrodites and males (61). Currently, studies estimate that the hermaphrodite connectome consists of 890 gap junctions, 6395 chemical synapses, and 1410 neuromuscular junctions (1) (62).

Interestingly, the sex of the animal can establish the synaptic connection and function of a neuron. Ascr#3 is also sensed by ADF, but only in males, and hermaphrodites which have been masculinized through expression of *fem-3,* which inhibits the sexual regulator gene, *tra-1* (63, 64). Neuronal activation of ADF by ascr#3 requires *mab*-3, which is naturally inhibited in hermaphroditic animals (63). As ADL is still activated in males, masculinized ADF inhibits the aversive response to ascr#3. This inhibition may be taking place via extrasynaptic connections, or direct serotonin signaling on downstream neuronal target of ADL (Figure 2B). Sex can also result in different physical circuits, where synapses between certain neurons are only present in males, and pruned in hermaphrodites (65). This highlights the necessity of investigating how sex results in specific connections underlying a behavioral circuit, not merely the requisite neuron, in order to generate a more complete functional connectome.

Modulation

Behavioral circuits are dependent on the state of the animal. While receptor expression profiles and the sex of the animal are set variables, more flexible states – largely the physiological state of the animal – shape and modulate these functional circuits. Sensory networks are altered by neuromodulators (neurotransmitters and neuropeptides) in a context specific manner; over varying distances and timescales. The effect of these modulations varies based on site of release and local concentration as governed by release, degradation, and reuptake of neuromodulators.

Serotonin (5-HT) has been shown to have a large role in behaviors related to foraging, egg laying, and locomotion, dependent on the presence or absence of food, as expression levels are correlated with being either fed or starved. For example, when food is present, 5-HT acts via GPA-11 to sensitize ASH to 30% octanol aversion (66). Interestingly, when dissecting the role of 5-HT, it was found that the site of release is important and can have opposite effects. 5-HT released from NSM sensitizes ASH to reverse quicker to 30% octanol. However, 5-HT released from ADF acts on ASH and results in shorter reversals, followed by forward locomotion (67). This highlights how a single neurotransmitter, in the same circuit, can give rise to different synaptic strengths and fine-tuned behavioral outputs, revealing that it is tantamount to not just consider neurotransmitters on a global scale. Moreover, the same stimulus does not necessarily utilize the same circuit at different concentrations. Different functional circuits are realized when animals are responding to 100% versus 30% octanol (66). At 100% octanol, ADL and AWB form electrical synapses onto command interneurons via GLR-1, are important

when animals are starved, whereas 30% octanol aversion, regardless of food presence, is only mediated by ASH (66).

Furthermore, timescales of stimulus detection appear to be programmed into the response. As seen in copper avoidance, a cross-talk inhibition circuit between ASI and ASH fine tunes the behavioral response, with ASH responding quickly and robustly in comparison to a slower, weaker response by ASI which inhibits further ASH activation (68). Whereas this is a short-term reciprocal inhibition state, long-term behavioral states also exist that shape functional circuits. For example, roaming and dwelling states in the presence of food alternate, and last for minutes at a time. This switch is achieved via two opposing neuromodulators, dwelling is promoted by serotonergic neurotransmitter signaling, whereas the roaming state is established by the neuropeptide PDF (Figure 2C) (69). Strikingly, this functional circuit acts in a seemingly unorthodox manner, as it defies classical circuit logic of sensory to motor organization: motor and interneurons modulate the activity of sensory neurons (69). This largely extrasynaptic, long-term timescale circuit has many potential inputs that can bias signaling of one state over another. Interestingly, the only overlap between these two circuits involves the interneuron AIY (69). Perhaps, the odor of food biases the switch between dwelling and roaming while on food.

In fact, an odor detection switch in local search behaviors intersects with AIY as well (70). AWC detects food depletion in a dose dependent way: as food is removed, AWC is disinhibited, allowing for inhibition of AIY (70, 71). Thus, it is plausible that short timescale detection by AWC recognizes changing concentrations of food, and relays this information to AIY, biasing a switch between the long-term roaming and dwelling states when on food. Together, functional connectomes can vary and take shape in drastically

different ways than wiring diagrams suggest, with particular synaptic importance being dictated by physiological states and timescales. Additionally, functional circuits do not work in isolation, the final behavioral output is a readout of the fine tuning of multiple functional circuits creating a functional connectome.

Functional circuits to one stimuli do not act alone, just as the internal state modulates the response to a particular cue, the presence of multiple stimuli are integrated into larger networks. One example of this is the "flip/flop" model of integration of contradictory "good" and "bad" stimuli. Previous research regarding multisensory integration in *C. elegans* highlights the importance of applying accessible methodologies of avoidance assays while considering neural circuitry more broadly such as through the connectome. For example, a study identifying a "flip-flop" circuit in *C. elegans* that is vital for the multisensory processing of aversive and attractive stimuli simultaneously would not have been as successful without this circuitry knowledge (72). Similarly, recent work on an inhibitory circuit essential for adaptive avoidance was able to explore intermediate functions because there was already a comprehensive understanding of synaptic connections and work on the functional mapping of said connections (68).

An example of this model occurs with high levels of repulsive quinine (sensed by ASH) and low levels of the attractant diacetyl (sensed by AWA) animals do not exhibit pharyngeal pumping. However, as the concentration of quinine is decreased, the pumping rate, in a steep sigmoidal fashion, increases, displaying a "flip" to increased pharyngeal pumping (72). Likewise, if quinine levels remain unchanged, a non-linear switch in pharyngeal pumping can be seen as diacetyl concentration increases (72). This flip/flop around a particular threshold requires 5-HT and tyramine signaling between the RIM, RIC,

and NSM interneurons via a serotonin channel (MOD-1) and tyramine receptor (SER-2) (72). Interestingly, animals that lack MOD-1 and SER-2 still show a decrease in pumping as quinine is increased, but in a linear fashion instead of a flip/flop, on/off switch at a particular threshold. Thus, these two sites of action for the neuromodulators are required for fine tuning the response around critical levels, but not for the overall integration of the two stimuli.

How integration of cues allows for the modulation of circuits can further be exemplified by looking at the tolerance of threats. Expectantly, *C. elegans* that are well fed are not willing to cross a high osmotic barrier to chemotax towards diacetyl, the risk is not worth reward. However, animals which are deprived of food will cross the same osmotic barrier, presumably as the risk is worth the reward (73). This modulation requires slow accumulation of tyramine, as it increases along with extended times of starvation, thereby desensitizing ASH to the osmotic stressor, and requires a few hours to reach a level which allows for the switch to decide to cross the osmotic barrier (73).

The aforementioned examples showcase the complexity underlying functional circuits, as there seem to be multiple levels of neuronal processing acting in parallel to finely adjust how the animal responds, including specific intercellular machinery allows for rapid adjustment of neuronal responses, thereby affecting the output, and these modulations can take place over long time scales - not merely minutes, but instead hours. Thus, to truly decipher a functional circuit, many different time points and stimulus concentrations would have to be investigated, as there is likely hidden information that acts at a deeper level.

Discussion and Future Directions

Nervous systems comprise of structurally interconnected neuronal networks and brain regions with complex connectivity patterns (74). As mapping and recording techniques become increasingly capable of capturing neural structure and activity across widely distributed circuits and systems, there is a growing need for new analysis tools and modeling approaches to make sense of these rich “big data” sets. *C. elegans* with its well characterized physical connections will provide for a better platform for more functional connectomic analysis to elucidate a better understanding of the connectivity patterns.

The future of functional connectomics will likely entail a strong push for rapid whole-brain imaging techniques, yet maintaining the detail of individual neurons. Currently, these techniques are not optimized in many organisms. In the case of *C. elegans,* using a technique to image a single neuron requires the animal to be moved into a separate testing environment. By this methodology, the worm is often reduced to a fixed space (75). This idea of imaging *in vivo*, yet removed from the natural environment rings to the same tune as MRI or other imaging techniques that require large pieces of equipment. On the other end of this spectrum however, exists techniques that do not even require a microscope. These lens-free methods include one such optofluidic microscope: *C. elegans* are still able to move around freely in solution while their behavior is monitored (76). This technique goes further than a behavioral assay, as it can obtain real time results from internal structures. Work has also moved more in the direction of *in silico* models. Driven by developments in software *C. elegans* have been modeled by programs like OpenWorm (77, 78).These types of modelling are rapidly growing alongside *in vivo*

research, as they can model both known behaviors (thereby predicting outcomes to an unknown stimulus), and predict behaviors based on trends and structural components from single neuron or pan-neuronal studies.

Developments in non-invasive techniques used to probe neural mechanisms have made reliable progress in the past several years. One method utilizes ultrasound to stimulate neural circuits in worms and other excitatory cells (79). The field of sonogenetics delivers ultrasound to manipulate the neural circuit through a variety of mediums. One method uses repeated exposure of low-pressure ultrasound with microbubbles while *C. elegans* remain on agar plates (80). Others turn to microfluidic chip devices to deliver a single, short pulse of ultrasound (81). As of 2016, the use of sonogenetics has been approved by the FDA to treat essential tremors in humans. This high-intensity focused ultrasound uses the mechanisms of MRI to map structures, before ablating damaged structures exacerbating tremors, typically localized in the thalamus (82). This, and treatments like it, are less invasive than typical surgical methods and are spurred by knowing how the structural connectome influences the functional.

In conclusion, connectomics (both structural and functional) are likely to expand significantly in coming years. Several large-scale national and international projects and consortia directed at brain science are underway, including the Human Connectome Project and the BRAIN initiative in the U.S. as well as the Human Brain Project in the E.U. (8, 10, 83). Given the rate of data generation, an interesting avenue is development of frameworks that can scale across different scales and systems, that can underpin and help make sense of “big brain data” (84).

Acknowledgements

We thank the *Caenorhabditis* Genetics Center (CGC), which is funded by the NIH Office of Research Infrastructure Programs (P40 OD010440). We thank the Srinivasan lab for critical comments on the manuscript and the Zhen lab for the EM picture of the worm connectome. This work was supported in by grants from the NIH R01DC016058 to J.S.

References

1. White JG, Southgate E, Thomson JN, Brenner S. The structure of the nervous system of the nematode Caenorhabditis elegans. Philos Trans R Soc Lond B Biol Sci. 1986;314(1165):1-340.
2. Glickstein M. Golgi and Cajal: The neuron doctrine and the 100th anniversary of the 1906 Nobel Prize. Cell. 2006;16(5).
3. Stephan KE, Kamper L, Bozkurt A, Burns GAPC, young MP, Kötter R. Advanced database methodology for the Collation of Connectivity data on the Macaque brain (CoCoMaq). Royal Society: Biological Sciences. 2001;356(1412).
4. Sporns O, Tononi G, Kötter R. The Human Connectome: A Structural Description of the Human Brain. PLoS Computational Biology. 2005;1(4):0245-51.
5. Seung S. Connectome: Houghton Mifflin Harcourt; 2012 [Connectomics, Neuroscience].
6. Seung HS. Towards functional connectomics. Nature. 2011;471:170-2.
7. Insel TR, Landis SC, Collins FS. The NIH BRAIN Initiative. Science. 2013;340(6133):687-8.
8. Yuste R, Bargmann C. Toward a Global BRAIN Initiative. Cell. 2017;168(6):956-9.
9. Ecker JR, Geschwind DH, Kriegstein AR, Ngai J, Osten P, Polioudakis D, et al. The BRAIN Initiative Cell Census Consortium: Lessons Learned toward Generating a Comprehensive Brain Cell Atlas. Neuron. 2017;96(3):542-57.
10. Van Essen DC, Smith SM, Barch DM, Behrens TEJ, Yacoub E, Ugurbil K, et al. The WU-Minn Human Connectome Project: an overview. NeuroImage. 2013;80:62-79.
11. Neylan TC. Frontal lobe function: Mr. Phineas Gage's famous injury. Journal of Neuropsychiatry Clinical Neuroscience. 1999;11(2):280-1.
12. Fang-Yen C, Gabel CV, Samuel ADT, Bargmann CI, Avery L. Laser Microsurgery in Caenorhabditis elegans. Methods in Cell Biology. 2012;107:177-206.
13. Chalfie M, Sulston J, White J, Southgate E, Thomson J, Brenner S. The neural circuit for touch sensitivity in Caenorhabditis elegans. Journal of Neuroscience. 1985;5:956-64.
14. Giurumescu CA, Chisholm AD. Cell Identification and Cell Lineage Analysis. Methods in Cell Biology. 2011;106:325-41.
15. Yuan J, Hyman B. Apoptotic and non-apoptotic roles of caspases in neuronal physiology and pathophysiology. Nat Rev Neuroscience. 2012;13(6):395-406.
16. Lauterbur P. Image Formation by Induced Local Interactions: Examples of Employing Nuclear Magnetic Resonance. Nature. 1973;242(5394):190-1.
17. Hagmann P, Kurant M, Gigandet X, Thiran P, Wedeen VJ, Meuli R, et al. Mapping Human Whole-Brain Structural Networks with Diffusion MRI. PLoS. 2007;2(7).

18. Colosimo C, Pontieri FE. Yawning in Parkinson's disease. Neurology. 1999;52(2).
19. Cornelia Bargmann, Marder E. From the connectome to brain function. Nature Methods. 2013.
20. Bargmann CI. Beyond the connectome: how neuromodulators shape neural circuits. Bioessays. 2012;34(6):458-65.
21. Gray JM, Hill JJ, Bargmann CI. A circuit for navigation in Caenorhabditis elegans. Proc Natl Acad Sci U S A. 2005;102(9):3184-91.
22. Wragg RT, Hapiak V, Miller SB, Harris GP, Gray J, Komuniecki PR, et al. Tyramine and octopamine independently inhibit serotonin-stimulated aversive behaviors in Caenorhabditis elegans through two novel amine receptors. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2007;27(49):13402-12.
23. de Bono M, Tobin DM, Davis MW, Avery L, Bargmann CI. Social feeding in Caenorhabditis elegans is induced by neurons that detect aversive stimuli. Nature. 2002;419(6910):899-903.
24. Hilliard MA, Bergamasco C, Arbucci S, Plasterk RH, Bazzicalupo P. Worms taste bitter: ASH neurons, QUI-1, GPA-3 and ODR-3 mediate quinine avoidance in Caenorhabditis elegans. The EMBO journal. 2004;23(5):1101-11.
25. Campbell JC, Chin-Sang ID, Bendena WG. Mechanosensation circuitry in Caenorhabditis elegans: A focus on gentle touch. Peptides. 2015;68:164-74.
26. Kaplan JM, Horvitz HR. A dual mechanosensory and chemosensory neuron in Caenorhabditis elegans. Proc Natl Acad Sci U S A. 1993;90(6):2227-31.
27. Hart AC, Kass J, Shapiro JE, Kaplan JM. Distinct signaling pathways mediate touch and osmosensory responses in a polymodal sensory neuron. The Journal of neuroscience : the official journal of the Society for Neuroscience. 1999;19(6):1952-8.
28. Hart AC, Sims S, Kaplan JM. Synaptic code for sensory modalities revealed by C. elegans GLR-1 glutamate receptor. Nature. 1995;378(6552):82-5.
29. Bastiani C, Mendel J. Heterotrimeric G proteins in C. elegans. WormBook. 2006:1-25.
30. Hodgkin A, Huxley A. A quantatitive description of membrane current and its application to conduction and excitation in nerve. Journal of Physiology. 1952;117(4).
31. Goodman MB, Lindsay TH, Lockery SR, Richmond JE. Electrophysiological Methods for C. elegans Neurobiology. Methods in Cell Biology. 2012;107:409-36.
32. Segev A, Garcia-Oscos F, Kourrich S. Whole-cell Patch-clamp Recordings in Brain Slices. Journal of Visual Experimentation. 2016;112.
33. Belliveau J, Jr DK, McKinstry R, Buchbinder B, Cohen M, Vevea J, et al. Functional mapping of the human visual cortex by magnetic resonance imaging Science. 1991;254(5032):716-9.
34. Berman MG, Jonides J, Nee DE. Studying mind and brain with fMRI. SCAN. 2006;1:158-61.
35. Deisseroth K. Optogenetics: Controlling the Brain with Light [Extended Version]. Scientific America. 2010.
36. Cho Y, Zhao CL, Lu H. Trends in high-throughput and functional neuroimaging in Caenorhabditis elegans. Wiley interdisciplinary reviews Systems biology and medicine. 2017;9(3).
37. Reilly DK, Lawler DE, Albrecht DR, Srinivasan J. Using an Adapted Microfluidic Olfactory Chip for the Imaging of Neuronal Activity in Response to Pheromones in Male C. Elegans Head Neurons. Journal of Visual Experimentation. 2017.
38. Whitesides G. The origins and the future of microfluidics. Nature. 2006;442:368-73.
39. Paraskevopoulos E, Kraneburg A, Herholdz SC, Bamidis PD, Pantev C. Musical expertise is related to altered functional connectivity during audiovisual integration. Proc Natl Acad Sci U S A. 2015;112(40):12522-7.
40. Duistermars B, Frye MA. Multisensory integration for odor tracking by flying Drosophila: Behavior, circuits and speculation. Commun Integr Biol. 2010;3(1):60-3.

41. Calvert GA, Brammer MJ, Bullmore ET, Campbell R, Iversen SD, David AS. Response amplification in sensory-specific cortices during crossmodal binding. NeuroReport. 1999;10(12):2619-23.
42. Zheng Z, Lauritzen JS, Perlman E, Robinson CG, Nichols M, Milkie D, et al. A Complete Electron Microscopy Volume of the Brain of Adult *Drosophila melanogaster*. Cell. 2018;174(3).
43. Schafer WR. The Worm Connectome: Back to the Future. Trends in neurosciences. 2018;41(11):763-5.
44. Ohyama T, Schneider-Mizell CM, Fetter RD, Aleman JV, Franconville R, Rivera-Alba M, et al. A multilevel multimodal circuit enhances action selection in *Drosophila*. Nature. 2015;520:633-9.
45. Ghosh DD, Nitabach MN, Zhang Y, Harris G. Multisensory integration in *C. elegans*. Current opinion in neurobiology. 2017;43:110-8.
46. Hillard MA, Bargmann CI, Bazzicalupo P. *C. elegans* Responds to Chemical Repellents by Integrating Sensory Inputs from the Head and the Tail. Curr Biol. 2002;12(9):730-4.
47. Metaxakis A, Petratou D, Tavernarakis N. Multimodal sensory processing in *Caenorhabditis elegans*. Open Biol. 2018;8(6).
48. Hart AC, Chao MY. From Odors to Behaviors in *Caenorhabditis elegans*. The Neurobiology of Olfaction: CRC Press/Taylor & Francis; 2010.
49. Walker DS, Vazquez-Manrique RP, Gower NJ, Gregory E, Schafer WR, Baylis HA. Inositol 1,4,5-trisphosphate signalling regulates the avoidance response to nose touch in Caenorhabditis elegans. PLoS Genet. 2009;5(9):e1000636.
50. Maricq AV, Peckol E, Driscoll M, Bargmann CI. Mechanosensory signalling in C. elegans mediated by the GLR-1 glutamate receptor. Nature. 1995;378(6552):78-81.
51. Mellem JE, Brockie PJ, Zheng Y, Madsen DM, Maricq AV. Decoding of polymodal sensory stimuli by postsynaptic glutamate receptors in C. elegans. Neuron. 2002;36(5):933-44.
52. Clark T, Hapiak V, Oakes M, Mills H, Komuniecki R. Monoamines differentially modulate neuropeptide release from distinct sites within a single neuron pair. PLoS One. 2018;13(5):e0196954.
53. Dong C, Reilly DK, Bergame C, Dolke F, Srinivasan J, Reuss SHv. Comparative Ascaroside Profiling of Caenorhabditis Exometabolomes Reveals Species-Specific (ω) and (ω − 2)- Hydroxylation Downstream of Peroxisomal βOxidation. Journal of Organic Chemistry. 2018;83:7109-20.
54. Jang H, Kim K, Neal SJ, Macosko E, Kim D, Butcher RA, et al. Neuromodulatory state and sex specify alternative behaviors through antagonistic synaptic pathways in C. elegans. Neuron. 2012;75(4):585-92.
55. Macosko EZ, Pokala N, Feinberg EH, Chalasani SH, Butcher RA, Clardy J, et al. A hub-and-spoke circuit drives pheromone attraction and social behaviour in C. elegans. Nature. 2009;458(7242):1171-5.
56. Brenner S. The Genetics of *Caenorhabsitis Elegans*. Genetics. 1973;77(1):71-94.
57. Hodgkin J. More Sex-Determination Mutants of *Caenorhabditis Elegans*. Genetics. 1980;96(3):649-64.
58. Sammut M, Cook SJ, Nguyen KCQ, Felton T, Hall DH, Emmons SW, et al. Glia-derived neurons are required for sex-specific learning in C. elegans. Nature. 2015;526:385-90.
59. Sulston J, Albertson D, Thomson J. The *Caenorhabditis elegans* Male: Postembryonic Development of Nongonadal Structures. Developmental Biology. 1980;78:542-76.
60. Loer CM, Kenyon CJ. Serotonin-Deficient Mutants and Male Mating Behavior in the Nematode *Ceanorhabditis elegans*. The Journal of neuroscience. 1993;13(12):5407-17.
61. Chute CD, Srinivasan J. Chemical mating cues in C. elegans. Seminars in cell & developmental biology. 2014;33:18-24.
62. Varshney LR, Chen BL, Paniagua E, Hall DH, Chklovskii DB. Structural Properties of the Caenorhabditis elegans Neuronal Network. PLoS Computational Biology. 2011;7(2).

63. Fagan KA, Luo J, Lagoy RC, Schroeder FC, Albrecht DR, Portman DS. A Single-Neuron Chemosensory Switch Determines the Valence of a Sexually Dimorphic Sensory Behavior. Curr Biol. 2018;28(6):902-14 e5.
64. White JQ, Nicholas TJ, Gritton J, Truong L, Davidson ER, Jorgensen EM. The Sensory Circuitry for Sexual Attraction in C. elegans Males. Curr Biol. 2007;17(21):1847-57.
65. Weinberg P, Berkseth M, Zarkower D, Hobert O. Sexually Dimorphic unc-6/Netrin Expression Controls Sex-Specific Maintenance of Synaptic Connectivity. Curr Biol. 2018;28(4):623-9 e3.
66. Chao MY, Komatsu H, Fukuto HS, Dionne HM, Hart AC. Feeding status and serotonin rapidly and reversibly modulate a Caenorhabditis elegans chemosensory circuit. Proc Natl Acad Sci U S A. 2004;101(43):15512-7.
67. Harris G, Korchnak A, Summers P, Hapiak V, Law WJ, Stein AM, et al. Dissecting the serotonergic food signal stimulating sensory-mediated aversive behavior in C. elegans. PLoS One. 2011;6(7):e21897.
68. Guo M, Wu TH, Song YX, Ge MH, Su CM, Niu WP, et al. Reciprocal inhibition between sensory ASH and ASI neurons modulates nociception and avoidance in Caenorhabditis elegans. Nat Commun. 2015;6:5655.
69. Flavell SW, Pokala N, Macosko EZ, Albrecht DR, Larsch J, Bargmann CI. Serotonin and the neuropeptide PDF initiate and extend opposing behavioral states in C. elegans. Cell. 2013;154(5):1023-35.
70. Chalasani SH, Chronis N, Tsunozaki M, Gray JM, Ramot D, Goodman MB, et al. Dissecting a circuit for olfactory behaviour in Caenorhabditis elegans. Nature. 2007;450(7166):63-70.
71. Calhoun AJ, Tong A, Pokala N, Fitzpatrick JA, Sharpee TO, Chalasani SH. Neural Mechanisms for Evaluating Environmental Variability in Caenorhabditis elegans. Neuron. 2015;86(2):428-41.
72. Li Z, Li Y, Yi Y, Huang W, Yang S, Niu W, et al. Dissecting a central flip-flop circuit that integrates contradictory sensory cues in C. elegans feeding regulation. Nat Commun. 2012;3:776.
73. Ghosh DD, Sanders T, Hong S, McCurdy LY, Chase DL, Cohen N, et al. Neural Architecture of Hunger-Dependent Multisensory Decision Making in C. elegans. Neuron. 2016;92(5):1049-62.
74. Sporns O. Connectome Networks: From Cells to Systems: Springer; 2016.
75. Chronis N, Zimmer M, Bargmann CI. Microfluidics for in vivo imaging of neuronal and behavioral activity in Caenorhabditis elegans. Nat Methods. 2007;4(9):727-31.
76. Kim SB, Bae H, Koo K-i, Dokmeci MR, Ozcan A, Khademhosseini A. Lens-Free Imaging for Biological Applications. Journal for Laboratory Automation. 2012;17(1):43-9.
77. Palyanov A, Khayrulin S, Larson SD. Application of smoothed particle hydrodynamics to modeling mechanism of biological tissue. Advances in Engineering Software. 2016;98:1-11.
78. Gleeson P, Cantarelli M, Currie M, Hokanson J, Idili G, Khayrulin S, et al. The OpenWorm Project: currently available resources and future plans. BMC neuroscience. 2015;16(Suppl 1):P141.
79. Kubanek J, Shukla P, Das A, Baccus SA, Goodman MB. Ultrasound Elicits Behavioral Responses through Mechanical Effects on Neurons and Ion Channels in a Simple Nervous System. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2018;38(12):3081-91.
80. Ibsen S, Tong A, Schutt C, Esener S, Chalasani SH. Sonogenetics is a non-invasive approach to activating neurons in Caenorhabditis elegans. Nat Commun. 2015;6:8264.
81. Zhou W, Wang J, Wang K, Huang B, Niu L, Li F, et al. Ultrasound neuro-modulation chip: activation of sensory neurons in Caenorhabditis elegans by surface acoustic waves. Lab on a chip. 2017;17(10):1725-31.

82. Fishman PS. Thalamotomy for essential tremor: FDA approval brings brain treatment with FUS to the clinic. Journal of therapeutic ultrasound. 2017;5:19-.
83. Amunts K, Ebell C, Muller J, Telefont M, Knoll A, Lippert T. The Human Brain Project: Creating a European Research Infrastructure to Decode the Human Brain. Neuron. 2016;92(3):574-81.
84. Sporns O. Making sense of brain network data. Nat Methods. 2013;10(6):491- 3.

Figure Legends

Figure 1. Since its discovery, *C. elegans* has played a pivotal role in expanding what is known about neural connections and the nervous system. The past 60 years have led to many developments in technologies, *green*, and advancements in neurological discoveries, *blue*, involving this microscopic nematode. Shown here are *C. elegans*-centric publications discussed in the review, additional information available in bracketed citation.

Figure 2. Differential use of neurons and pathways to create functional circuits. **A)** The same neuron, ASH, responds to two different stimuli, using different intracellular signaling pathways and post synaptic targets to give rise to the same behavioral avoidance output. Osmotic stress is sensed and signaled via *osm-10* and targets the NMDA-type receptor *nmr-1* whereas nose touch utilizes *itr-1* and the glutamate receptor *glr-1*. **B)** Gender and type of synapse can govern behavioral output to the same stimulus. ADL senses the ascaroside, ascr#3, and in solitary hermaphrodites (high *npr-1*) results in avoidance via electrical synapses. However, hermaphroditic animals with low *npr-1* activity dampen or even reverse the valence of response to ascr#3 by the hub and spoke gap junction circuit, specifically ASK and RMG. Gender also shapes the response to ascr#3. Males are also able to detect ascr#3 via the masculine *mab-3* expressing state of ADF and are attracted to the compound. It is likely that ADF is opposing the ADL promoted avoidance response either via input to command interneurons or the first layer amphid interneurons it synapses with. **C)** Functional circuits may be shaped by both neurotransmitters and neuropeptides and by short and long timescales. When animals are on

food, they display long term behavioral dwelling or roaming states which are triggered by serotonin and PDF, respectively. Interestingly, we see that functional connections can be extrasynaptic and defy sensory to motor circuit logic as HSN/NSM serotonin inhibits ASI in this behavior. Furthermore, despite the complex network of connections (dotted lines) between these neurons, we see that these states are largely governed by a few, extrasynaptic signaling connections. Lastly, the switch between dwelling and roaming is seemingly spontaneous, as AIY is the only neuron that is involved in both states, we propose that it may be acting as a switch via input from AWC, which can detect food odor levels and relay information to AIY.