



Published in final edited form as:

*Curr Opin Neurobiol.* 2017 April ; 43: 110–118. doi:10.1016/j.conb.2017.01.005.

## Multisensory integration in *C. elegans*

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### Abstract

Multisensory integration is a neural process by which signals from two or more distinct sensory channels are simultaneously processed to form a more coherent representation of the environment. Multisensory integration, especially when combined with a survey of internal states, provides selective advantages for animals navigating complex environments. Despite appreciation of the importance of multisensory integration in behavior, the underlying molecular and cellular mechanisms remain poorly understood. Recent work looking at how *Caenorhabditis elegans* makes multisensory decisions has yielded mechanistic insights into how a relatively simple and well-defined nervous system employs circuit motifs of defined features, synaptic signals and extrasynaptic neurotransmission, as well as neuromodulators in processing and integrating multiple sensory inputs to generate flexible and adaptive behavioral outputs.

Sensory systems are continuously barraged with input from the environment. Nervous systems collect this information, process it to identify relevant events and objects in the surroundings, and interpret these representations of the outside world to navigate the environment. This immensely complex neural function is further convoluted by the richness and variability of the sensory cues in the natural world. Multisensory integration, especially when combined with a survey of the internal physiological state, provides selective advantages to animals navigating complex environments. In humans and other mammals, a key motif of multisensory integration is the “top-down” control of perception, wherein the primary sensory cortex activity is modulated and/or controlled by feedback from higher-order regions [1–5]. A number of neurological diseases, such as autism spectrum disorder, sensory-processing disorder, schizophrenia, bipolar disorder and ADHD, display abnormal processing and integration of sensory information due to alterations in multisensory circuits or changes in neuromodulatory circuits [6–10]. However, analysis of neural mechanisms of multisensory processing and integration in human beings is limited to psychological and functional imaging studies, and cannot elucidate detailed cellular and molecular mechanisms. In contrast, the studies of mammalian model organisms allow manipulation of

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cellular and molecular function in the nervous system. In one recent study, for example, a combination of optogenetics, electrophysiology, and psychophysical approaches was used to establish that top-down projections from the frontal cortex modulate sensory information processing in the primary visual cortex by engaging local microcircuits [5]. However, this level of detailed cellular and molecular analysis has not been applied to the dissection of neural mechanisms underlying perceptual decision-making, perhaps because of the difficulty of combining sophisticated experimental manipulations with complex behavioral decision paradigms in freely-moving animals.

Studies on multisensory integration in the nematode *C. elegans* have begun to bridge this gap. *C. elegans* is uniquely amenable to the combination of sophisticated cellular and molecular manipulations with physiological and behavioral measurements in freely-moving animals. The *C. elegans* nervous system contains only 302 identified neurons of defined synaptic connectivity [11], and many of those neurons can be readily targeted for cell-specific genetic manipulation. Despite the relatively simple nervous system, worms respond to a variety of sensory modalities, including smell, taste, touch, high osmolarity, temperature, oxygen, light, and responses to these stimuli in isolation are relatively well-characterized, robust, and quantifiable [12–21]. Most importantly, many studies demonstrate that the features of mammalian sensory integration, including both the control of the internal state on multisensory decision-making and the involvement of neuromodulators, are present in the worm, suggesting that these fundamental processes are conserved. In this review, we will first describe the behavioral paradigms that have been employed in demonstrating multisensory integration in *C. elegans*. We will then attempt to summarize common themes that have emerged from characterizing the mechanisms underlying multisensory integration with these behavioral paradigms, followed by a discussion on the functional consequences of multisensory integration in behavior.

### ***C. elegans* performs multisensory integration**

All animals navigate in complex natural environments that contain both dangers and valuable sources, such as predators and food. Many of these sources are represented by more than one sensory cue. Thus, animals need to process multiple sensory cues in order to make decisions that benefit survival and reproduction. Behavioral paradigms used to investigate multisensory integration in *C. elegans* have been developed by taking advantage of the sensitivity of the worms to an array of sensory modalities. These paradigms include: 1) Expose the worms to two sensory cues of opposing valence and ask the animals to make a decision between the cues [22,23]; 2) Expose the worms to a single sensory stimulus that is paired with a contextual cue, such as ambient temperature or food availability [24–27]. Using these paradigms, it has been shown that integrated behavioral responses are regulated by several distinct, but overlapping neuronal and circuit mechanisms, which we will review as the following.

## Cellular and circuit mechanisms underlying multisensory integration

### Integration of multiple sensory cues at the level of sensory neurons

**Polymodal sensory neurons**—Sensory neurons in *C. elegans* have the capability to be what is referred to as “polymodal” [28]. This has been demonstrated for many of the sensory neurons using optical imaging, laser/genetic ablation and behavioral analysis, such as the ASH sensory neurons that have been shown to respond to an array of aversive stimuli of different modalities, including mechanosensation, noxious stimuli, pH, quinine, and food odors to direct sensorimotor responses [13,19,20,27,29–58]. Previous studies show that ASH neurons coordinate these responses by using different synaptic signals. During aversive responses to osmolarity and nose touch, distinct synaptic pathways and glutamate receptors are required for escape behavior [35,42]. Glutamate receptors GLR-1 and GLR-2 are required for nose touch, but only play a modest role in mediating responses to hyperosmolarity. Conversely, the NMDA-like receptor NMR-1 is dispensable for nose touch responses, but important for osmolarity responses [42]. Taken together, this specific example demonstrates one mechanism whereby ASH neurons are capable of specifying the behavioral response to distinct sensory stimuli by coordinating different synaptic outputs to mediate aversion [42]. Overall, while it is relatively poorly understood how the polymodal neurons process multiple inputs simultaneously, the studies on the ASH neurons provide an example of how multisensory integration in individual sensory neurons generate reliable and cue-specific inputs to downstream circuits.

**Multiple sensory neurons combinatorially encode sensory cues**—While a number of *C. elegans* sensory neurons are polymodal, many sensory neurons are often employed together to decode a complex sensory environment [24,27,34,46,59]. For example, carbon dioxide is a common sensory cue that may signal the presence of bacterial food sources to *C. elegans*. However, the behavioral response of *C. elegans* to CO<sub>2</sub>, sensed by sensory neurons AFD and BAG, among others, is not fixed, and the extent of repulsion depends on ambient oxygen concentrations, temperature, and nutritional state in a way that is dependent on other sensory neurons like URX [24,27]. These findings suggest that multiple sensory neurons together generate a more complete representation of the environment before converging on an appropriate response to environmental CO<sub>2</sub> level. Hygrosensation also requires integration of multiple sensory cues. Worms respond to humidity with FLP and AFD sensory neurons that are known to sense mechanical stimuli and temperature, respectively, to coordinate locomotory behaviors that drive hygrosensation [46]. Furthermore, even within the same sensory modality multiple sensory neurons are used to respond to complex cues. For example, olfactory preference between the smells of two bacterial foods, *E. coli* OP50 and *Pseudomonas aeruginosa* PA14, requires the function of the olfactory sensory neurons AWB and AWC, which sense repulsive versus attractive odorants, respectively. Both sensory neurons respond to the food odors, which are likely blends of volatile metabolites [34].

Sensory neurons can also regulate the function of one another to mediate sensorimotor behaviors. For example, small increases in salt concentration stimulate the ASE sensory neurons, which are sufficient to direct chemotaxis, while the AWC neurons are not involved.

Interestingly, upon larger increases in salt concentrations, the ASE neurons recruit the AWC neurons as interneurons via the action of insulin-like peptides to coordinately regulate chemotaxis [38,60]. Another study shows that responses to medium concentration of a food odor, benzaldehyde, engages coordinated activity of the AWA and AWC neurons, which sense benzaldehyde, and the ASE and AWB sensory neurons, which act downstream of these primary sensory neurons [39]. Crosstalk between sensory neurons and the plasticity of their identity and function found therein represent a model by which flexible assembly of neuronal networks produces appropriate behavioral responses to unisensory or multisensory cues. As another example of this model, a recent study identifies neuropeptide-dependent changes in the signaling of olfactory circuits after mechanosensation is compromised. These remodeled circuits then lead to changes in food odor-dependent locomotory behaviors [61]. Together these studies, by revealing interactions among sensory circuits, demonstrates another potential cellular mechanism underlying multisensory integration.

### Neural circuit motifs that mediate multisensory behavior

Characterization of circuit motifs that underlie multisensory integration, such as coincidence detectors and hub-and-spoke circuits, have revealed mechanistic insights into the role of the wiring diagram in this process.

**Coincidence detector**—While the *C. elegans* nervous system contains 13 pairs of amphid sensory neurons that respond to a variety of stimuli, many of these sensory neurons chemically or electrically connect with the same set of interneurons, including the AIA, AIB, AIY and AIZ neurons [11]. This high degree of convergence provides an anatomical basis for these interneurons to act as coincidence detectors to receive and process inputs from multiple sensory neurons that respond to simultaneously presented sensory cues of different modalities [22,23,48,62–65]. For example, when worms are simultaneously confronted with an attractive odorant diacetyl sensed by the chemosensory neurons AWA and an aversive stimulant  $\text{Cu}^{2+}$  sensed by the polymodal sensory neurons ASH, they make a behavioral decision between approach and avoidance. Several signaling molecules, including a guanylyl cyclase GCY-28, regulate this behavioral decision in the interneuron AIA, which is postsynaptic of ASH and electrically connected with AWA [11,23,65]. It is conceivable that GCY-28 acts in AIA to process convergent sensory inputs that represent the attractant and the repellent [65]. In another paradigm, animals confronted with an aversive odorant octanol (primarily sensed by ASH neurons) decide to retreat and move away from the odorant or initially retreat from the odorant but then continue to move forward, depending on the presence or absence of food [48,63]. The AIB interneuron controls this decision by integrating sensory-evoked glutamatergic synaptic signals from ASH and from the food odor sensing neuron, AWC. In this case, the two convergent signals coordinately regulate the activity of AIB and the convergence of the two synaptic signals allows AIB to produce an integrated output to downstream circuits that control locomotion [48,63]. Overall, interneurons like AIB can modulate locomotion during responses to an array of sensory inputs through distinct but overlapping pathways in a state-dependent manner that can be modulated by specific contexts [62,66].

**Hub-and-spoke circuit**—Pheromones play important roles in worm behavior by signaling the population density or the presence of mates [67–73]. Worms are able to sense pheromones through sensory neurons, such as ASK and ADL [71–73]. Both ASK and ADL, as well as a few other sensory neurons including an oxygen sensing neuron URX, connect with a single inter/motor neuron pair RMG through gap junctions, forming a hub-and-spoke circuit that is able to integrate multiple sensory inputs. The activity state of the hub neuron RMG regulates aggregation by mediating the activity of spoke neurons, such as ASK. High activity level of RMG potentiates the response of ASK to pheromone, resulting in attraction among individual animals [73]. These findings reveal a common type of circuit motif where a central hub neuron is able to regulate an integrated behavioral response to multiple sensory cues sensed by the spoke neurons by regulating the activity of the spoke neurons. A similar “hub-and-spoke” circuit has been identified to regulate nose touch response that drives worms to reverse when encountering an object during forward movement [37,45]. Here, three pairs of sensory neurons (spokes), which all respond to gentle mechanical stimulation to the nose, are connected via gap junctions with the hub neuron, the interneuron RIH [32,37,45]. The spoke neurons in this circuit could regulate each other, indicating an information flow among the spoke neurons. Together, these findings reveal several mechanisms through which a common circuit motif can generate an integrated response to multiple sensory inputs. Interestingly, multilayer circuit motifs also mediate multisensory integration processing in *D. melanogaster* fly larvae, in which integration of mechanosensory and nociceptive inputs span multiple layers of sensory processing circuitry to enhance escape response behaviors [74].

### **Neuromodulators regulate multisensory integration by modulating the state of neural circuits**

Neuromodulators, such as biogenic amines and neuropeptides, are involved in many studies on multisensory integration in *C. elegans*. Because neuromodulators can operate extrasynaptically at a distance on short or long timescales, they functionally connect neuronal networks in a way that is unpredictable with the anatomical connectome alone [75]. Therefore, neuromodulators present distinct advantages over synaptic neurotransmitters in multisensory information processing. In the above mentioned paradigm where a worm makes a decision between approaching the attractive odorant diacetyl and avoiding the repulsive tastant copper, the potential coincidence detector, AIA, is regulated by a secreted protein HEN-1 that is produced by another interneuron, AIY, probably through a receptor tyrosine kinase SCD-2 [23,65]. The ability of neuromodulators to facilitate interactions between neurons that have no anatomical connection not only allows neuromodulation to occur within a neuronal class, such as HEN-1 signaling between interneurons AIY and AIA, but also cross different neuronal types. For example, the biogenic amine tyramine that is secreted by inter/motor neuron RIM regulates the decision to cross a hyperosmotic barrier, sensed by the sensory neuron ASH, to reach a source of food odor by extrasynaptically modulating ASH activity [22]. This type of modulation can occur in a feedback loop [76,77]. For example, the major olfactory sensory neuron AWC and one of the postsynaptic interneurons AIA reciprocally regulate each other via two different neuropeptides [76]. This feedback loop of peptidergic signals modulates sensory dynamics to generate flexible behavioral outputs when animals are stimulated with a complex pattern of cues.

An important function of neuromodulation is to regulate neuronal property in a context-dependent manner. The context can be external, such as the sensory environment of an animal, or internal, such as the state of a neural circuit. Integrating these contexts with sensorimotor response generates flexible behavioral outputs [22,25,26,48,57,58,62,63,65,66,73,75,78–86]. For example, in the above-mentioned study in which worms were confronted with a hyperosmotic barrier preventing it from reaching the food odor diacetyl, one hour of food deprivation significantly increases threat tolerance, promoting the decision to cross the osmotic barrier. In this decision-making process, the function of RIM is to provide tyraminerpic inputs to ASH based on metabolic state. The tyramine levels represent the internal state, because food deprivation mimics the behavioral effect of reducing the tyraminerpic signaling in a way that is dependent on the low level of tyramine. The modulation of the osmosensitivity of ASH controls perception of the hyperosmotic barrier and thereby the multisensory threat-reward decision [22]. Further, tyramine and octopamine signaling can act as one of the off-food signals that modulate aversive responses to a repellent octanol. In this case, tyramine from RIM inhibits the ASH-mediated avoidance behaviors by modulating a multi-sensory network that includes sensory neurons ASI and interneuron AIA [79,81]. Thus, the RIM-dependent tyraminerpic modulation can act as an off-food response that fine-tunes the multisensory circuit in different ways to generate flexible behavioral outputs. Remarkably, it was recently shown that tyraminerpic/octopaminergic neurons suppress aversion to bitter-tasting foods in starved *D. melanogaster* flies [80].

On the other hand, the presence of food also translates important information through neuromodulators to provide contextual cues to the nervous system, which can influence multisensory integration and decision-making. Serotonin levels increase when the worm is on food [25,48,63]. Either an increased serotonin level or the presence of food inhibits the activity of one of the first layer interneurons AIB and can modulate the sensitivity of the sensory neuron ASH [48,58]. In these contexts, a worm exhibits increased threat tolerance by resuming forward movement after initial retreat from the repulsive odorant octanol [48,63]. Dopamine also provides information about food and is known to regulate ASH sensitivity to a variety of noxious stimuli [26]. During a decision-making to cross an aversive tastant copper to reach an attractive food odor, loss of the D2-like dopamine receptor decreases threat tolerance, while loss of the D1-like dopamine receptors increases it [87]. In addition, dopamine also signals the pattern of the food experienced by a worm to modulate search behavior through an extrasynaptic signal that regulates the activity of the underlying neural network [88]. The neuromodulatory regulation in *C. elegans* multisensory decision-making is similar to the internal-state-dependent “top-down” control of perception by higher-order brain regions in humans and other mammals [1–5]. Using *C. elegans* for analysis of neural mechanisms in the context of multisensory decision are advantageous due to fewer technical constraints. The recent findings that we described here reveal how *C. elegans* integrates multisensory inputs and converts these inputs into a decision.

## Functional significance of multisensory integration

Overall, we have reviewed a number of multisensory integration behaviors and possible underlying mechanisms in *C. elegans* (Figure 1). Together, these studies suggest that



multisensory integration can take place at different layers of the nervous system using synaptic and non-synaptic communication under various contexts. Here, we propose several important functional consequences of multisensory integration. First, integrating sensory cues of multiple modalities allows animals to better extract relevant information in a complex environment to facilitate decision-making. Second, integrating multiple inputs with circuit motifs that contain particular signal processing properties or through neuromodulation greatly increases the flexibility of behavioral outcomes. In addition, we also propose that multisensory integration may share some of the underlying mechanisms with learning, which allows animals to generate experience-dependent changes in neuronal function and behavior. During learning, an animal often needs to pair one sensorimotor response with another or to pair a sensorimotor response with a contextual cue. The process of pairing may use similar cellular mechanisms as multisensory integration, although prolonged pairing in learning often leads to a lasting effect in behavior [89]. Together, we propose that multisensory integration contributes to the adaptive value of flexible behavioral outcomes in an environment that is complex in both contents and spatial-and-temporal features.

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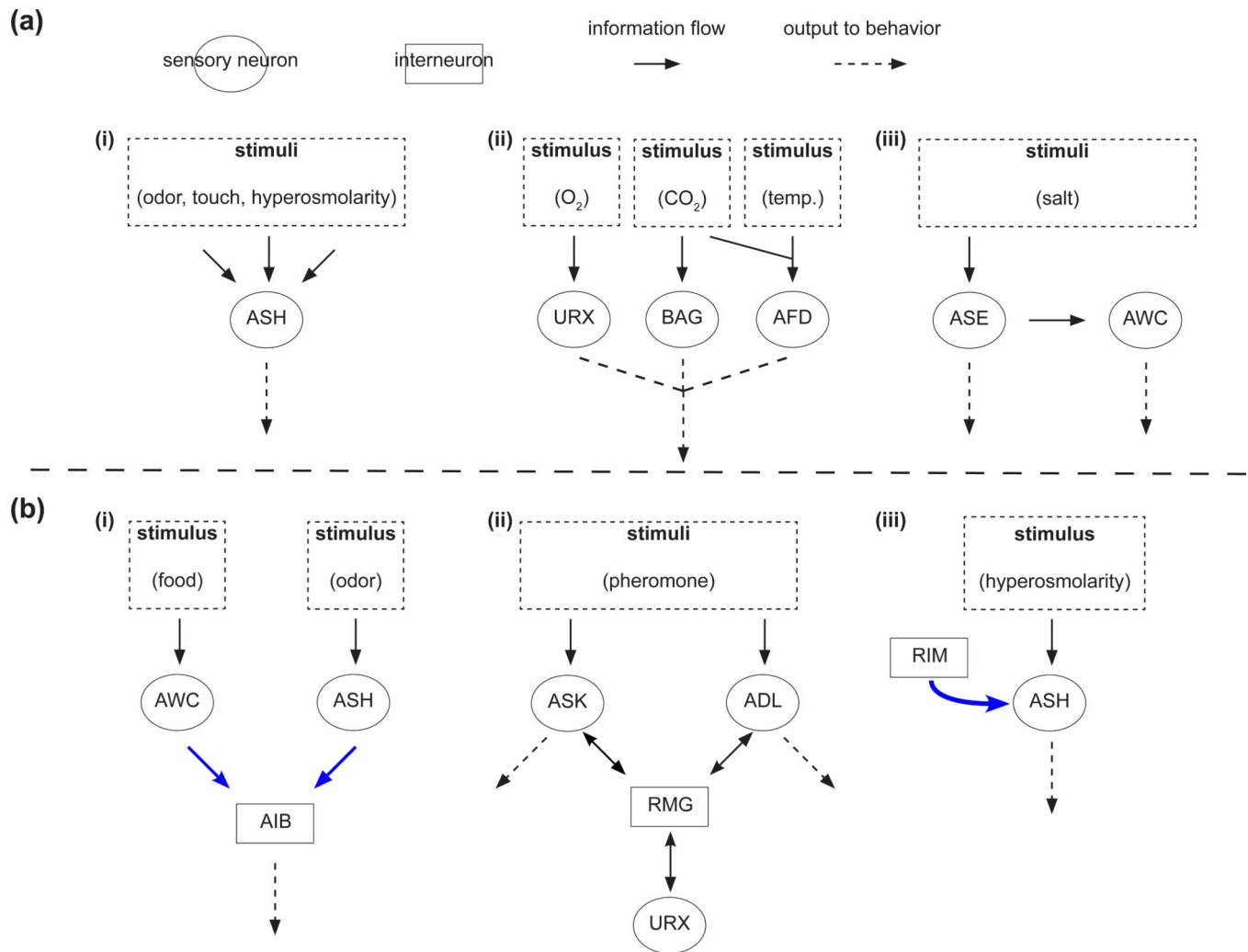
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neuron, through gap junctions to form a “hub-and-spoke” circuit capable of integrating multiple sensory inputs to control a complex behavior. The activity state of RMG is critical for social aggregation. The high activity state of RMG promotes aggregation by potentiating the response of ASK to pheromones. This study reveals a common motif in the worm connectome and demonstrates mechanisms whereby multiple sensory cues can be processed and integrated to generate coherent behavioral outputs. [PubMed: 19349961]

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**Figure 1. Different mechanisms of multisensory integration in *C. elegans***

**(a)** Different mechanisms by which multisensory inputs converge at cellular sites. (i) Polymodal sensory neurons are themselves capable of integrating multiple sensory stimuli; (ii) sensory neurons work with one another to form a more complete representation of the multisensory environment, then use that information to direct responses to specific sensory stimuli; (iii) sensory neurons recruit one another, even one not ordinarily sensitive to the sensory stimulus, as an interneuron to generate a more appropriate response to the sensory stimulus. Examples of sensory stimuli and neurons are provided. Arrows represent information flow, and depict known functional relationships between neurons indicated by genetic, anatomical, behavioral, or physiological analysis.

**(b)** Different circuit motifs underlying multisensory decision making. (i) Interneurons direct behavioral responses to simultaneously presented multisensory stimuli by integrating inputs from sensory neurons that respond to distinct unisensory stimuli present in the environment; (ii) a “hub” interneuron interact with “spoke” sensory neurons to direct behavioral responses to stimuli detected by the sensory neurons; (iii) in a “top-down” fashion, an interneuron regulates a multisensory decision by tuning the sensitivity of a sensory neuron. Arrows in



blue denote characterized transmitter signaling systems like glutamate, peptides, or monoamines.

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