Thermosensory Neurons Mediate Tactile-Dependent Locomotion Modulation in *C. elegans*

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A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2025

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Program Authorized to Offer Degree:

Molecular and Cellular Biology

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Abstract

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Behavioral plasticity is the ability of animals to modify their behavior, a capacity essential for survival in constantly changing environments. This flexibility arises from experience-dependent changes in neuronal activity that modulate the flow of information through neural circuits. These changes are supported by molecular and circuit mechanisms that promote behavioral adaptation by modulating a wide range of neuronal functions, including excitability, synaptic strength, and connectivity. Disruption of these mechanisms can contribute to a wide range of neurological and psychiatric disorders that severely impair behavior. Despite their importance, the fundamental cellular and molecular principles of neuronal function and behavioral plasticity remain poorly understood, partly because the complexity of mammalian systems makes it difficult to dissect these mechanisms at the cellular and molecular levels. To bypass these limitations, neuroscientists have turned to simpler vertebrate and invertebrate animal models to gain insight into the molecular and circuit mechanisms that enable behavioral plasticity. One such model is the nematode *Caenorhabditis elegans*, which offers powerful genetic tools, and a well-mapped nervous system ideally suited for dissecting these mechanisms. In this dissertation, I investigated the molecular and circuit mechanisms that enable context-dependent behavioral plasticity in the nematode *C. elegans*. Using behavioral assays and genetic tools, I

uncovered a novel role for the thermosensory neuron AFD in modulating locomotion based on tactile experience across different contexts. Genes in a cGMP signaling pathway are required in AFD for tactile-dependent modulation. Although these genes have previously been implicated in AFD's role in thermosensation, our findings indicate that they function in tactile modulation independently of thermal signaling. This conclusion is supported by the observation that tactile-dependent modulation persists even in the absence of AFD's thermosensory apparatus, suggesting that AFD does not act as a sensory neuron in this context. Rather, its function appears to depend on electrical synapses with the AIB interneuron, indicating that AFD's connectivity is essential for its role in tactile-dependent behavior. Together, these findings reveal a previously unrecognized circuit logic in which AFD contributes to behavioral plasticity by integrating information from other sensory modalities. This work advances our understanding of how molecular signaling and circuit architecture shape behavior and highlights *C. elegans* as a powerful model for dissecting the molecular basis of neuronal plasticity.

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1. Molecular and Circuit Mechanisms Underlying Behavioral Plasticity

Behavioral plasticity is the ability of animals to modify their behavior, an essential survival strategy for adapting to ever-changing environments. Scientific interest in how animals adjust their behavior dates back thousands of years. One of the earliest written accounts appears in Aristotle's *Historia Animalium*, a work describing factual knowledge about animal anatomy and behavior. Aristotle noted that animals can change their behavior in response to environmental changes, often gaining a survival advantage [1]. Today, understanding the mechanisms that support behavioral plasticity is crucial, as they underlie key aspects of mental health, including memory consolidation, contextual integration, emotional regulation, and adaptive decision-making [2–5]. These capacities rely on the brain's ability to integrate sensory input, past experience, and internal state, functions that are often impaired in neurological and psychiatric disorders such as autism, depression, and schizophrenia [6–9]. From Aristotle's early observations to modern neuroscience, the study of behavioral plasticity continues to provide critical insights into the biological foundations of behavior and mental health.

Over the past century, studies across a wide range of species have revealed diverse forms of behavioral plasticity. Researchers have learned that animals modify their behavior based not only on the current environment, but also on memories of past experiences, enabling them to better navigate their surroundings and respond to internal and external cues [10–14]. For instance, the prior dietary experience of animals shapes their behavior in ways that help them identify food-rich areas and develop strategies to navigate toward them. When food is scarce, animals typically increase their locomotor activity to explore wider areas, with the direction of movement fine-tuned by previously learned associations between sensory cues and food availability [15–21]. These and other findings have shown that behavioral modifications often depend on individual

history, motivating neurobiological research into the principles that explain how animals retrieve information from experience to shape behavioral output. By leveraging the genetic tractability and experimental accessibility of model organisms, researchers have made significant discoveries about the cellular and molecular mechanisms that underlie behavioral plasticity.

1.1 The Study of Neurons in Animal Models Reveal the Basis of Behavioral Plasticity

It has taken thousands of years to arrive at our current understanding of the relationship between the nervous system and behavioral plasticity. Millennia ago, the philosopher and physician Claudius Galenus demonstrated that the brain plays a central role in controlling body movement, suggesting that behavior arises from the brain's ability to direct physical actions [22,23]. Yet it was only a century ago that Ramón y Cajal discovered the brain is composed of individual cells, called neurons, that communicate through specialized junctions called synapses, providing the first anatomical evidence that neuronal communication underlies the brain's ability to modulate behavior [24]. Decades later, the development of electrophysiological techniques enabled scientists to directly link the electrical activity of neurons to observable behaviors. Notably, Erik Kandel's work in the sea slug *Aplysia* showed that motor neuron activity correlated with gill withdrawal upon tactile stimulation [25]. Repeated stimulation led to diminished motor neuron firing and reduced gill retraction, demonstrating that prior experience can be encoded in the electrical properties of neurons [26–28]. These findings have since driven decades of research into biological mechanisms facilitating neural plasticity and their role in behavior.

Research into how the nervous system enables behavioral plasticity has identified several strategies for modulating neuronal activity. One key strategy involves changes in chemical synapses, the primary means of communication between neurons. The synapse is a specialized structure where presynaptic neurons release neurotransmitters in response to

stimulation, which bind to postsynaptic receptors that modulate the neuron's electrical activity. Experience-dependent changes in neurotransmitter release and postsynaptic responsiveness, collectively known as synaptic plasticity, are mediated by a variety of mechanisms [29,30]. Increases in synaptic strength, known as potentiation, can result from elevated neurotransmitter release [31], an increased number of receptors [32,33], or enhanced receptor responsiveness [34,35]. In contrast, decreases in strength, known as depression, arise from reduced release [36], fewer receptors [37–39], or diminished receptor sensitivity [40,41]. Synaptic plasticity spans timescales from seconds to weeks, enabling neurons to encode information essential for learning, memory, and behavioral adaptation. For example, long-term synaptic plasticity has been linked to the ability of animals, including flies and mice, to form lasting associations with sensory stimuli through associative conditioning. In mice, associative learning is accompanied by increased presynaptic neurotransmitter release, enhanced postsynaptic receptor responsiveness, and activity-dependent trafficking of glutamate receptors to the synaptic membrane [42–44]. In the fruit flies, *D. melanogaster*, plasticity in the mushroom body, a center for olfactory learning, involves changes in synaptic strength, neurotransmitter release, and receptor localization [45–48]. These findings underscore its role as a fundamental mechanism by which the nervous system encodes experience to guide future behavior.

In addition to modulating synaptic communication, neuronal pruning facilitates the remodeling of physical structures within the nervous system, shaping circuit connectivity. Pruning refers to the selective elimination of synapses, axons, dendrites, or even entire neurons, and it plays a central role in restructuring neural networks to support behavioral adaptation [49]. Pruning can eliminate neuronal communication in response to changes neural activity, refining circuit architecture to improve the efficiency and fidelity of information flow. While pruning is prominent during development, when excessive axonal projections are pruned due to insufficient activity, this process continues into adulthood, allowing the nervous system to

fine-tune neuronal connectivity in response to experience [50,51]. For example, various training paradigms in mice induce changes in the number of synapses, suggesting that pruning contributes to the dynamic refinement of neural circuits based experience-dependent neuronal activity [52–55]. Pruning has been observed across diverse species, including nematodes, fruit flies, and zebrafish [56–58]. In these organisms, pruning occurs both during development and through experience-dependent mechanisms in adulthood, contributing to the refinement of neural circuits. Its presence across phyla highlights pruning as an evolutionarily conserved strategy for shaping functional nervous systems, expanding the repertoire of activity-dependent processes through which experience reshapes circuit function to support behavioral adaptation.

A key feature of neuronal modulation is its ability to persist over time by engaging transcriptional regulation. Activity-induced calcium influx initiates signaling cascades involving second messengers that activate transcription factors, which in turn regulate genes controlling synaptic growth, neurotransmitter release, receptor trafficking, and structural remodeling [59,60]. One well-studied example is CREB, a transcription factor conserved from nematodes to mammals. For instance, In the nematode, *C. elegans,* CREB mediates the adaptation of a thermosensory neuron in response to prolonged thermal stimulation by initiating gene transcription changes within minutes of prolonged thermal stimulation [61]. In mice, CREB is essential for long-term synaptic plasticity and memory formation. Disruption of CREB function in mice impairs long-term synaptic potentiation and reduces performance in associative learning tasks, such as fear and reward conditioning [62–65]. While CREB has received considerable attention, several other transcription factors also contribute to activity-dependent gene regulation, highlighting the diversity of transcriptional responses to neuronal activity [66]. Transcriptional regulation enables neurons to convert transient patterns of activity into lasting changes in gene expression that support long-term modulation of neuronal activity. Such

sustained transcriptional responses are driven by upstream signaling pathways, many of which rely on second messengers to link neural activity to changes in gene expression.

Fluctuations in intracellular levels of second messengers, including cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), and calcium ions, occur in response to neuronal activity, enabling them to propagate signals within the cell and regulate downstream processes in an activity-dependent manner. These second messengers typically initiate signaling cascades that culminate in the activation of protein kinases or phosphatases, which regulate ion channel activity, drive receptor trafficking, modulate cytoskeletal dynamics for structural remodeling, and influence gene expression [67–69]. For example, increased intracellular calcium levels activate calmodulin and calcium-dependent kinases, which regulate multiple aspects of neuronal function, including the trafficking of neurotransmitter receptors at the synapse and the structural remodeling of the synapse itself [70,71]. Similar mechanisms are observed with other second messengers to facilitate behavioral plasticity. Both cGMP and cAMP, for instance, are implicated in the adaptation of sensory responses across species. Mechanistically, cAMP activates protein kinase A (PKA), while cGMP activates protein kinase G (PKG); both kinases phosphorylate downstream targets that modulate ion channel activity, neurotransmitter release, and gene expression. In fruit flies, cAMP signaling plays a central role in behavioral plasticity by modulating synaptic strength across multiple neural circuits. The cAMP-dependent protein kinase (PKA) pathway is crucial for learning and memory, arousal, and sensory adaptation [72–74]. In mammals, second messenger pathways contribute to complex behaviors that require learning and memory, such as fear conditioning [75,76]. These examples illustrate how second messengers operate as integrative hubs, translating transient synaptic activity into long-lasting changes in neuronal function and highlighting their role in signaling networks that support behavioral adaptation.

Because the signaling pathways that regulate neuronal plasticity are conserved across species, animal models have been instrumental in uncovering the cellular and molecular mechanisms that support behavioral flexibility. Research in organisms ranging from nematodes and fruit flies to mice and zebrafish has revealed how synaptic strength, circuit structure, and gene expression are shaped by experience to produce adaptive behaviors. These models offer unparalleled access to tools for genetic manipulation, circuit dissection, and live imaging, making them essential for linking molecular mechanisms to circuit-level dynamics and behavior. Among these organisms, *C. elegans* is particularly well suited for behavioral studies due to its rich repertoire of strategies for modifying behavior in response to prior experiences with odors, temperature, touch, and hunger, among others [77–85]. The worm's behavioral repertoire, combined with its genetic tractability, has enabled researchers to dissect the relationship between genes and behavior, leading to the identification of conserved genetic pathways that modulate neuronal activity and contribute to behavioral plasticity.

1.2 A Map of Neuronal Wiring Reveals How Circuit Architecture Supports Behavior.

To this day, the *C. elegans* connectome remains the most complete map of neuronal connections in any animal, describing the synaptic wiring of neurons across the entire body. The *C. elegans* connectome was reconstructed by collecting electron micrographs of serial cross-sections along the worm's body and tracing neuronal outlines, allowing scientists to map all neurons and their synaptic connections [86]. The connectome revealed that the *C. elegans* nervous system consists of 302 neurons, broadly divided into three classes: sensory neurons that detect environmental stimuli; interneurons, which neither detect sensory stimuli nor synapse onto muscle; and motor neurons that form direct synapses onto body-wall muscles [86,87]. Having a complete map of the worm's neural network has enabled neurobiologists to investigate how

network activity encodes behavioral output, how it is shaped by experience, and the molecular mechanisms that support this modulation.

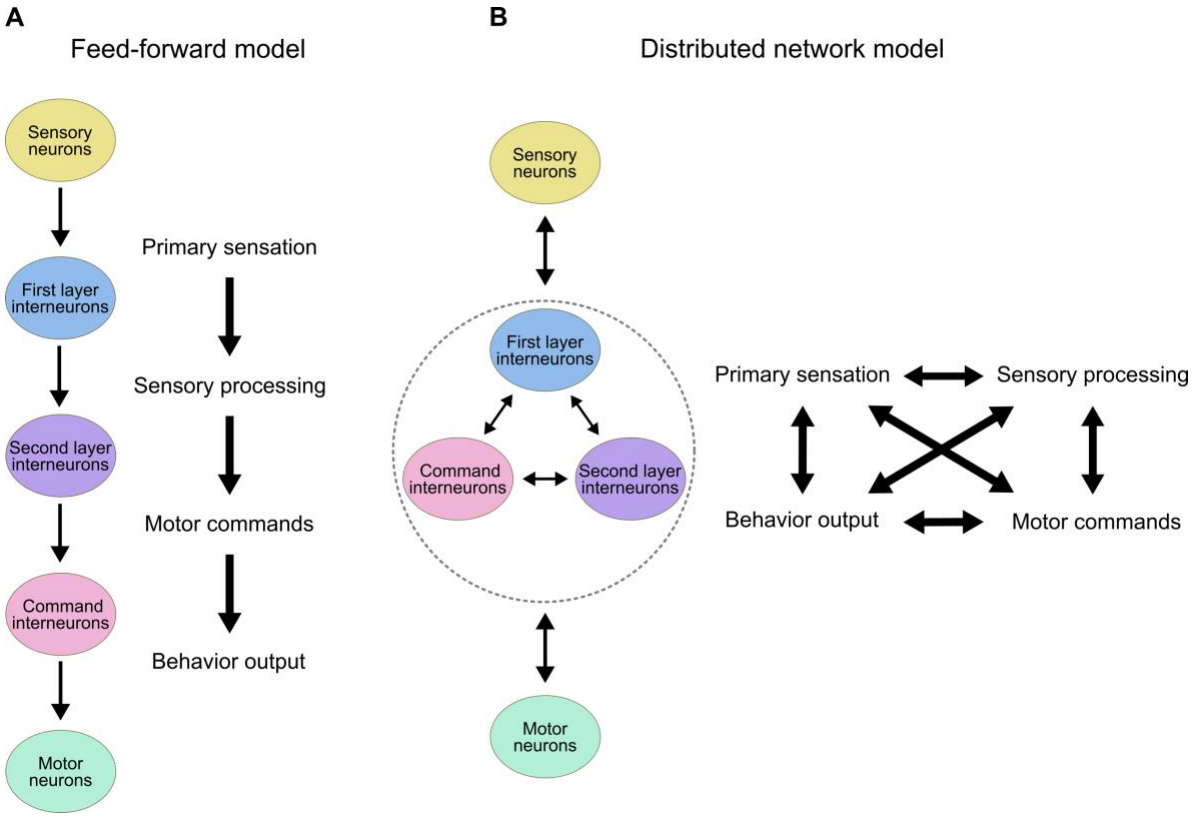


Figure 1.1. Schematic representation of feed-forward (A) distributed network (B) models of sensorimotor integration for behavior modulation. Adapted from Kaplan et al., 2018.

The organization of the *C. elegans* nervous system led neuroscientists to propose that it operates through feed-forward circuits. In this model, behavior is modulated by sensory neurons that are activated by environmental stimuli and transmit signals to interneurons, which process the input and relay it to motor neurons that drive locomotion (Fig. 1.1A). Behavioral studies support this framework, showing that sensory-evoked behaviors require intact synaptic connections between sensory neurons and interneuron networks, as ablation of neurons along the pathway from sensory to motor neurons disrupts the behavioral response [77,79,88–94]. However, advances in whole-brain imaging have revealed that sensory-evoked changes in brain activity cannot be fully explained by a feed-forward model [95]. First, in the absence of sensory

stimulation, interneurons exhibit spontaneous activity that correlates with the worm's behavioral state [96–98]. Rather than merely relaying sensory signals, these interneurons appear to integrate feedback from motor circuits reflecting ongoing behavior, suggesting a bidirectional flow of information within the network. Second, sensory neuron activation also correlates with changes in the activity of neurons that are not part of the same synaptic pathway, suggesting that extra-synaptic mechanisms further contribute to brain-wide modulation [99]. These findings have led to an updated understanding of how behavior is encoded in the *C. elegans* nervous system. The current view is that behavioral information is distributed across the brain and that sensory stimuli bias this distributed activity to shape behavioral output (Fig. 1.1B).

The *C. elegans* connectome provides a comprehensive map of structural connections but lacks information about how those connections are used under different conditions. Evidence

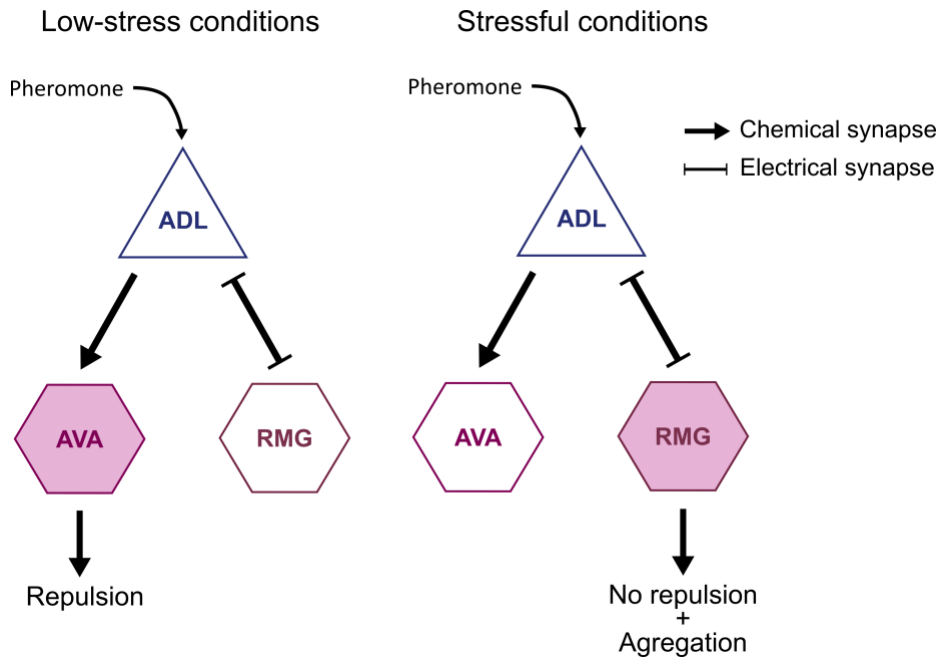


Figure 1.2. Diagram illustrating how stress modulates ADL-RMG/AVA circuit output to control pheromone avoidance. In low-stress conditions (left), ADL's chemical synapse with AVA promotes avoidance behavior. In contrast, under stress (right), ADL's electrical synapse with interneuron RMG is essential to dampen the avoidance response. This model exemplifies how internal states dynamically reconfigure circuit output to generate diverse behavioral responses.

that circuit function cannot be inferred from static connectivity maps comes from studies of divergent synaptic pathways that originate from the same neuron but produce distinct behavioral outcomes depending on how that neuron engages its downstream partners [100].
For example, the chemosensory neuron ADL detects pheromones and can promote repulsion, or dampen it, depending on the environmental conditions of the worms [101–104]. ADL forms chemical synapses with the command interneuron AVA to promote aggregation in well-fed animals grown in low-stress (control) conditions, while electrical synapses with the interneuron RMG are essential to dampen pheromone repulsion under stressful conditions such as starvation or hypoxia [105–107] (Fig. 1.2) These findings show that ADL uses distinct synaptic outputs to generate context-dependent behaviors, indicating that the *C. elegans* connectome is not a static blueprint. They underscore the field's growing interest in understanding how molecular mechanisms modulate information flow through neuronal circuits based on experience.

Building on foundational insights from *C. elegans*, connectome reconstruction in other model organisms such as the fruit fly (*D. melanogaster*) and the larval zebrafish (*D. rerio*) has revealed how conserved circuit principles are elaborated in more complex nervous systems. These connectomes have uncovered canonical circuit motifs, such as parallel sensory pathways and recurrent loops, also observed in *C. elegans*, which support sensory processing, learning, and adaptive behavior [108–111]. The connectomes of higher-order organisms exhibit a layered architecture, with information flowing from sensory neurons through intermediary processing layers to motor command neurons [109,111]. Extensive interconnectivity between layers is thought to enable distributed processing of behavioral information across neuronal networks, supporting flexible and context-dependent responses [108,109]. Although the development of these connectomes is relatively recent, and further analysis is needed to understand how connectivity contributes to behavioral plasticity, advances in technologies such as whole-brain activity

mapping in zebrafish hold promise for bridging the gap between neuronal activity, connectivity, and behavior [108]. Ultimately, these efforts echo a lesson first learned from *C. elegans*: while a connectome can map the potential paths of information flow, it cannot by itself explain how neural circuits change to generate adaptive behavior. To truly understand brain function, we must investigate how activity patterns arise within a structural framework—and how they are dynamically shaped by experience and internal state.

1.3 cGMP Modulates Neuronal Function to Support Behavioral Plasticity in C. elegans.

A major goal in uncovering how experience modulates the flow of information in neural circuits is to identify the intracellular signaling mechanisms that allow neurons to adapt their activity to environmental and internal conditions. In my research, described in Chapter 3, I identified genes involved in a cGMP signaling pathway that are required for context-dependent behavioral plasticity in *C. elegans*, consistent with the role of cGMP as a key signaling molecule in neuronal modulation. Indeed, decades of research have shown that cGMP dynamically modulates neuronal excitability and synaptic strength, allowing neurons to adjust information flow based on experience [112,113]. By tuning how neurons respond to input and communicate with downstream partners, cGMP signaling supports flexible, context-dependent behavior. Given the importance of cGMP signaling in behavioral plasticity and its relevance to my research, this section provides an overview of current knowledge on how cGMP modulates neuronal activity to facilitate behavioral plasticity in *C. elegans*.

cGMP is a key second messenger in sensory neurons, mediating the transduction of environmental cues. This process typically begins with guanylyl cyclases (GCs) that synthesize cGMP in response to external signals. cGMP then activates cyclic nucleotide–gated (CNG)

channels, leading to cation influx, neuronal depolarization, and neurotransmitter release. In *C. elegans*, cGMP mediates the transduction of sensory stimuli from various modalities, such as olfaction, oxygen sensing, and thermosensation [114–118]. For example, AWC is an olfactory neuron activated by odorants such as benzaldehyde, isoamyl alcohol, and butanone [119,120]. These odorants are detected by distinct G protein–coupled receptors (GPCRs), whose activation signals converge on regulation of a single GC, ODR-1, and multiple phosphodiesterases (PDEs) to decrease cGMP productions, consequently reducing CNG channel activity to hyperpolarize the neuron [121,122] (bottom panel Fig. 1.3). These transduction mechanisms are conserved across species and have been identified in insect and mouse olfactory neurons, as well as in human photoreceptors [123–125]. In photoreceptors, light-activated GPCRs activate Gα subunits that modulate cGMP synthesis and degradation, which in turn influences CNG channel permeability allowing the photoreceptor between the presence and absence of photons [123,126,127] (top panel Fig. 1.3). This mechanism allows light to regulate the excitability of photoreceptor cells and thereby shape the visual signals transmitted to the optic nerve. Beyond sensory transduction, elevated cGMP levels can also activate additional signaling pathways that modulate neuronal activity to facilitate behavioral adaptation.

The activation of cGMP-dependent protein kinases (PKGs) plays a central role in modulating neuronal activity to support long-term behavioral adaptation. By phosphorylating target proteins and transcription factors, PKGs mediate both rapid posttranslational changes in protein function and long-lasting shifts in neuronal state through transcriptional regulation [128–133]. In *C. elegans*, PKGs are encoded by two genes, *egl-4* and *pkg-2*, both implicated in regulating neuronal activity [132,134,135]. While the function of *pkg-2* remains less well understood, *egl-4* has been extensively studied. EGL-4 modulates neuronal excitability in response to elevated cGMP by phosphorylating key signaling proteins, including ionotropic and metabotropic receptors at the synapse. For example, in the nociceptive neuron ASH, EGL-4 phosphorylates the regulators

of G protein signaling RGS-2 and RGS-3, reducing the sensitivity of a GPCR responsible for detecting a noxious chemical and thereby decreasing aversive behavior [136]. Sustained cGMP production also triggers EGL-4 translocation to the nucleus, where it phosphorylates transcription factors to reprogram gene expression and promote long-lasting neuromodulation. In olfactory neurons, nuclear EGL-4 activity reprograms gene expression to mediate olfactory adaptation in response to prolonged odor exposure by reducing odor sensitivity [137–140]. The role of PKGs in *C. elegans* is conserved across animal species, including mammals, where cGMP signaling modulates neuronal activity underlying processes such as memory formation, circadian rhythm regulation, and sensory adaptation [128,141–144]. Together, these findings highlight PKGs as integrators of cGMP signaling that link intracellular molecular events to long-lasting changes in neuronal function and behavior.

Emerging evidence suggests that, in addition to its cell-autonomous activity, cGMP can diffuse through electrical synapses to influence neighboring cells, enabling cell-nonautonomous modulation of neuronal activity. Electrical synapses are gap junctions that create direct cytoplasmic connections between neurons, permitting the bidirectional flow of ionic currents and small molecules [145]. While best known for transmitting electrical signals via ionic currents [146,147], these synapses also allow diffusion of signaling molecules like cGMP, which can modulate physiology in adjacent cells [148–150]. In *C. elegans*, nonautonomous cGMP signaling occurs in the ASH-ASK-ADF circuit, where cGMP produced in ASK and ADF diffuses into ASH through gap junctions to suppress aversive responses to noxious stimuli such as quinine [150–153]. Within ASH, cGMP acts through the cGMP-dependent protein kinase EGL-4 and cyclic nucleotide–gated channels to reduce neuronal excitability and dampen aversive behavioral output [136]. These findings highlight how electrical synapses extend the influence of intracellular signals beyond individual cells, enabling circuit-level modulation.

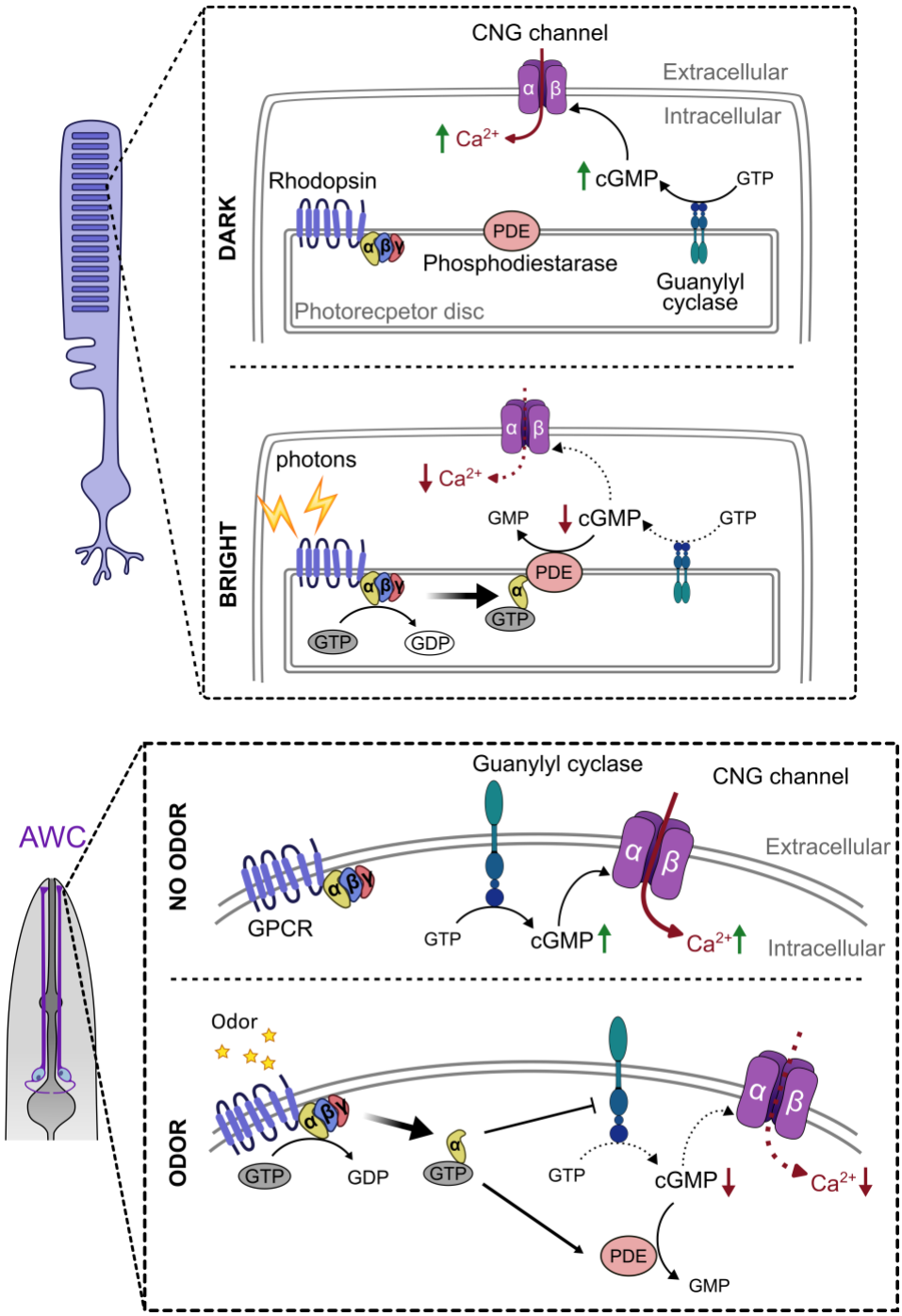


Figure 1.3. Conserved cGMP/CNG signaling in vertebrate photoreceptors and *C. elegans* olfactory neuron, AWC. In vertebrate photoreceptors (top), GCs produce cGMP in the dark to keep CNG channels open. Light activates rhodopsin and a G protein cascade that stimulates PDEs, reducing cGMP and closing CNG channels. A similar mechanism operates in AWC (bottom): in the absence of odor, GCs maintain cGMP to keep CNG channels open. Odor detection activates GPCR signaling that lowers cGMP, either by inhibiting GCs or activating PDEs, leading to channel closure. In both systems, sensory input modulates cGMP to control CNG channel activity and drive rapid, reversible changes in excitability.to drive rapid and reversible changes in neuronal excitability.

Together, these findings illustrate how behavioral plasticity arises from molecular signals that modulate the activity of neuronal circuits, allowing experience to be integrated to produce adaptive behavior outputs. This principle has been particularly well demonstrated in *C. elegans*, whose compact nervous system, mapped connectome, and genetic tractability have made it a uniquely powerful model for uncovering these mechanisms. Among its neurons, the thermosensory neuron AFD has emerged as a key site for studying how sensory experience is integrated to shape behavior output. In the following chapters, I focus on how AFD contributes to context-dependent behavior modulation, both in its canonical role in thermosensation and in a newly identified, thermosensation-independent role that highlights its versatility as a circuit integrator.

2. A Thermosensory Neuron Modulates Context-Dependent Behavior

Worms have developed a wide range of behaviors that allow them to navigate toward favorable environments using contextual information gained from prior experience. One such strategy is thermotaxis—the ability to migrate toward familiar temperatures. This behavior was first described in the 1970s, when researchers were searching for sensory-evoked behaviors that could be used to screen for genetic mutations affecting neural function. Hedgecock and Russel observed that worms placed on a surface with a temperature gradient consistently migrated toward regions near their cultivation temperature and remained within a narrow range around it [154]. These findings demonstrated that worms integrate their thermal experience into locomotor behavior to seek familiar environments. Importantly, the identification of mutants with defective thermotaxis laid the foundation for decades of research into the cellular, molecular, and circuit mechanisms that support experience-based modulation of behavior.

2.1 Thermosensory Neuron AFD Encodes Temperature Context.

It was not until two decades after thermotaxis was first described that a series of studies, building on the mutants identified in the 1970s, uncovered the sensory neuron pair AFD as essential for this behavior. In the 1980s, scientists began investigating the relationship between genes that disrupt behavior and the morphology of sensory neurons. By comparing serial cross-section micrographs of wild-type and mutant worms, they found that one mutation known to impair thermotaxis, *ttx-1(767)*, disrupted the formation of ciliary structures at the dendritic endings of AFD, suggesting that AFD was critical for thermotaxis [155]. About a decade later, laser ablation experiments confirmed that removing AFD severely disrupts the worm's ability to migrate to preferred temperatures [156]. Together, these findings established AFD as a key neuron involved in integrating thermal context to guide behavior.

Functional studies have shown that AFD contributes to thermotaxis by detecting changes in temperature. Electrophysiological recordings and calcium imaging using GCaMP have shown that Ca^{2+} influx triggers AFD depolarization when worms are exposed to a rising temperature ramp [118,157,158]. The dendritic ending of AFD is responsible for detecting thermal stimuli, as shown in experiments where the dendrite was severed from the cell body. In this setup, Ca^{2+} influx was observed in the isolated dendrite, but not in the cell body, when the neuron was exposed to a rising temperature ramp. These findings established AFD as the primary thermosensory neuron in *C. elegans*, capable of transducing environmental thermal signals into neuronal activity.

In addition to its thermosensory function, AFD exhibits adaptability that allows it to encode thermal context. Functional studies have shown that AFD depolarizes only when a defined temperature threshold is exceeded [118,157]. Importantly, this threshold is not fixed: it shifts depending on the worm's recent thermal experience. For example, in worms cultivated at 20 °C,

AFD's threshold is around 20 °C, but brief pre-conditioning at 18 °C or 25 °C lowers or raises the threshold, respectively [118,157,159]. These findings confirmed AFD as a thermosensory neuron and revealed its plasticity, which allows it to modulate locomotor behavior based on thermal context.

2.2 cGMP Synthesis Facilitates Temperature Sensing.

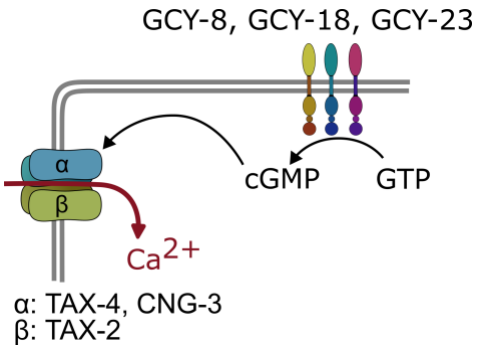


Figure 2.1. cGMP signaling pathway mediates temperature sensing in AFD thermosensory neurons. In AFD, temperature-dependent activation of GCs drives cGMP production, which activates the CNG channels to induce Ca^{2+} influx.

The second messenger cGMP plays an essential role in modulating AFD thermosensory activity. The Ca^{2+} dynamics observed during temperature ramps depend on cGMP synthesis mediated by the GCs *gcy-8*, *gcy-18*, and *gcy-23*, which act synergistically to generate fast and reliable temperature-evoked responses [160]. Null mutations in any one of these genes only partially disrupt thermally evoked Ca^{2+} responses, resulting in slower dynamics and reduced amplitude. However, loss of two or more of these GC genes severely impairs AFD's ability to respond to thermal stimuli preventing worms from migrating to preferred temperature [161,162]. The cGMP produced by these cyclases activates cyclic nucleotide-gated (CNG) channels, composed of the alpha-subunit TAX-4 and the beta-subunit TAX-2 [163], leading to increased intracellular Ca^{2+} (Fig. 2.1) Mutations in either *tax-4* or *tax-2* abolish thermally evoked Ca^{2+} responses in AFD and disrupt thermotaxis, underscoring the essential role of cGMP signaling in thermotransduction [118].

Thermally evoked cGMP is thought to activate kinases that, in turn, modulate AFD's activation threshold. Using the cGMP fluorescent reporter FlincG3, which exhibits dynamics similar to GCaMP during rising temperature ramps, researchers showed that either *gcy-18* or *gcy-23* alone is sufficient to drive rapid adaptation of the threshold for cGMP synthesis in AFD,

mirroring the threshold shifts observed in Ca^{2+} responses [159]. To identify downstream effectors of cGMP involved in threshold adaptation, researchers examined the CNG channel subunits expressed in AFD. They found that, an alpha subunit of CNG channels, CNG-3, plays a specific role in adaptation, as *cng-3* null mutants still respond to thermal stimuli—unlike *tax-2* or *tax-4* mutants [118]—but fail to adapt their thermal threshold. Furthermore, a point mutation at a predicted phosphorylation site in CNG-3 phenocopied the null mutant, suggesting that kinase-mediated phosphorylation is required for adaptation [159]. Consistently, worms lacking the cGMP-dependent kinases EGL-4 or PKG-2 are unable to adjust the threshold at which Ca^{2+} levels increase, despite cGMP levels rising at an appropriately adapted threshold [159]. The fact that PKG mutants do not affect cGMP production, but affects Ca^{2+} influx, supports a model in which cGMP modulates the activation threshold of CNG channels via phosphorylation. Together, these findings establish a mechanistic model of how molecular signaling mediates adaptive plasticity in the thermosensory neuron AFD.

2.3 AFD Modulates Distinct Aspects of Thermotaxis Through Different Synaptic Partners.

AFD transmits thermal signals to multiple downstream interneurons, including AIY, AIZ, and AIB, which process this information to guide temperature-dependent behaviors. Thermotaxis in *C. elegans* includes three main behavioral categories: movement toward warmer temperatures (thermophilic), movement toward cooler temperatures (cryophilic), and movement within a preferred temperature range (isothermal) [154,164]. Each behavior depends on distinct AFD synaptic partners: AIY is critical for thermophilic and isothermal behaviors [156,165]; AIZ is required for cryophilic behavior [166–168]; and AIB contributes to thermophilic behavior, although its role is less critical than that of AIY [169]. The importance of these connections for thermotaxis was first demonstrated through cell ablation experiments informed by the *C. elegans* connectome.

Subsequent molecular studies have shown that modulation of synaptic strength at these connections plays a pivotal role in determining behavioral outcomes.

2.4 A New Role for AFD Independent of Thermosensation

In this study, we demonstrate that AFD plays a novel role in tactile-mediated, context-dependent locomotion modulation, thereby expanding its function beyond temperature sensing. The mechanisms underlying this role differ markedly from those involved in thermosensation. First, context-dependent modulation requires distinct cGMP signaling components. Second, while the microvilli-rich sensory endings of AFD are essential for thermosensation, they are dispensable for context-dependent modulation. Third, this modulation depends on mechanosensory neurons and on electrical synapses between AFD and the AIB interneuron, which connects to mechanosensory circuits. Finally, disruption of *inx-7* and *inx-10* genes, encoding gap junction protein innexins in AFD and AIB, impairs context-dependent locomotion modulation. Remarkably, restoring AFD–AIB connectivity via engineered Cx36 electrical synapses rescues this function. Collectively, our findings uncover an unconventional mechanism by which AFD mediates behavior modulation based on tactile experience.

3. Manuscript: AFD Thermosensory Neurons Mediate Tactile-Dependent Locomotion Modulation in C. elegans

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3.1 Abstract

Sensory neurons drive animal behaviors by detecting environmental stimuli and relaying information to downstream circuits. Beyond their primary roles in sensing, these neurons often form additional synaptic connections outside their main sensory modality, suggesting broader contributions to behavior modulation. Here, we uncover an unexpected role for the thermosensory neuron AFD in coupling tactile experience to locomotion modulation in *Caenorhabditis elegans*. We show that while AFD employs cGMP signaling for both thermotaxis and tactile-dependent modulation, the specific molecular components of the cGMP pathway differ between these two processes. Interestingly, disrupting the dendritic sensory apparatus of AFD, which is essential for thermotaxis, does not impair tactile-based locomotion modulation, indicating that AFD can mediate tactile-dependent behavior independently of its thermosensory apparatus. In contrast, ablating the AFD neuron eliminates tactile-dependent modulation, pointing to an essential role for AFD itself, rather than its sensory dendritic endings. Further, we find tactile-dependent modulation requires the AIB interneuron, which connects AFD to touch circuits via electrical synapses. Removing innexins expressed in AFD and AIB abolishes this modulation, while re-establishing AFD-AIB connections with engineered electrical synapses restores it. Collectively, these findings uncover a previously unrecognized function of AFD beyond thermosensation, highlighting its influence on context-dependent neuroplasticity and behavioral modulation through broader circuit connectivity.

3.2 Introduction

Adaptive behavioral responses require animals to interpret sensory signals in the context of prior experiences, enabling effective responses to changing environmental conditions. For example, rodents associate contextual cues, such as odors and colors, with rewards or punishments, leading to context-dependent adjustments in approach or avoidance behaviors

[170,171]. Disruptions in the biological processes underlying this plasticity, as seen in neurological and psychiatric disorders, often result in impaired behavior modulation based on prior sensory experiences [172–176]. Recognizing the critical role of context-dependent behavioral modulation for survival and well-being, ongoing research focuses on uncovering the neuroplastic mechanisms responsible for these adaptive processes.

Studies across diverse organisms reveal that animals, regardless of nervous system complexity, integrate prior sensory experiences to shape behavior [177–184]. In the nematode *C. elegans*, which has only 302 neurons, locomotion strategies are modified by prior exposure to stimuli such as odors, touch, light, and starvation, demonstrating robust adaptive behaviors [185–192]. The wide range of adaptive behaviors, combined with the well-mapped *C. elegans* nervous system and abundant genetic tools, have empowered the discovery of fundamental circuit mechanisms and molecular pathways underlying context-dependent behavioral modulation [193–197].

The *C. elegans* sensory system comprises 60 ciliated sensory neurons specialized in detecting chemical, thermal, mechanical, and olfactory stimuli [198,199]. Each sensory neuron has a dendritic apparatus that converts specific environmental signals into neuronal activity, which drives signal flow through downstream networks of interneurons and motor neurons, ultimately shaping behavior [200–202]. Among these, AFD, a pair of bilaterally symmetric left-right neurons, serves as the primary thermosensory neurons required for thermotaxis (movement toward the a preferred temperature) [85]. The dendritic endings of AFD, enriched with microvilli, serve as the primary site for thermal detection. Even when detached from the cell body, these dendritic endings retain calcium responsiveness to temperature stimuli [157]. Moreover, genetically disrupting these sensory endings abolishes the Ca^{2+} response in AFD cell bodies to temperature changes and eliminates thermotaxis, underscoring their essential role in thermal sensing [203–205].

The second messenger cyclic guanosine monophosphate (cGMP) plays a central role in modulating AFD thermosensory activity. Temperature changes in AFD neurons trigger intracellular Ca^{2+} dynamics, with Ca^{2+} levels rising during warming and falling during cooling [118,157,158]. This response relies on cGMP synthesis mediated by the guanylyl cyclases (GCs) *gcy-8*, *gcy-18*, and *gcy-23*, which have overlapping but distinct roles. Specifically, *gcy-8* is essential for generating thermally evoked Ca^{2+} responses in AFD [160,161], whereas *gcy-18* and *gcy-23* act synergistically to sustain temperature sensing [161,162]. The cGMP produced by these cyclases activates cyclic nucleotide-gated (CNG) channels, comprised of alpha-subunits TAX-4 and beta-subunits TAX-2, resulting in increases in intracellular Ca^{2+}. Mutations in either *tax-4* or *tax-2* abolish thermally evoked Ca^{2+} responses in AFD and disrupt thermotaxis, underscoring the importance of cGMP signaling for AFD-mediated thermosensation [118].

AFD transmits thermal signals to several downstream interneurons, including AIY, AIZ, and AIB, which process this information to guide temperature-dependent behaviors. Thermotaxis in *C. elegans* encompasses three categories: movement toward warmer temperatures (thermophilic), movement toward cooler temperatures (cryophilic), and movement within a preferred temperature range (isothermal) [154,164]. Each behavior type depends on distinct AFD synaptic partners: AIY is critical for thermophilic and isothermal behaviors [156,165]; AIZ is specific for cryophilic behavior [166–168]; and AIB also supports thermophilic behavior, though its role is less critical than that of AIY [169]. These findings demonstrate the functional specificity of synaptic partners of AFD neurons in shaping distinct temperature-dependent behaviors.

In this study, we demonstrate that AFD plays a novel role in tactile-mediated, context-dependent locomotion modulation, thereby expanding its function beyond temperature sensing. The mechanisms underlying this role differ markedly from those involved in thermosensation. First, context-dependent modulation requires distinct cGMP signaling components. Second, while the microvilli-rich sensory endings of AFD are essential for thermosensation, they are dispensable for context-dependent modulation. Third, this modulation depends on

mechanosensory neurons and on electrical synapses between AFD and the AIB interneuron, which connects to mechanosensory circuits. Finally, disruption of *inx-7* and *inx-10* genes, encoding gap junction protein innexins in AFD and AIB, impairs context-dependent locomotion modulation. Remarkably, restoring AFD–AIB connectivity via engineered Cx36 electrical synapses rescues this function. Collectively, our findings uncover an unconventional mechanism by which AFD mediates behavior modulation based on tactile experience.

3.3 Results.

***C. elegans* displays distinct locomotion in identical environments after exploring different physical structures.**

To investigate the impact of prior experience on *C. elegans* behavior, we first allowed worms to explore chambers with distinct pillar designs (exploration zone, Fig. 1A) before recording their locomotion in identical assay zones (red dashed-line boxes, Fig. 1A). We hypothesized that if behavior were solely driven by immediate sensory input, worms in identical assay zones would exhibit similar locomotion. Conversely, if prior experience, such as exposure to different physical features in the exploration zones, influences behavior, then worms should display distinct locomotor patterns even in the identical assay zones. To test these hypotheses, we employed two types of microfluidic chambers: uniform and binary (Fig. 1A). Both chamber types include an identical assay zone. Once worms enter this zone, their immediate environment is the same regardless of the chamber. However, before reaching the assay zone, worms explore surrounding areas with pillar designs that differ in size and spacing. This design allowed us to assess how prior exposure to different physical contexts impacts locomotion in an otherwise identical setting.

Worms were allowed to explore the chambers for 60 minutes before their behavior was recorded in the assay zones. We found that worms in the uniform chamber assay zone

exhibited locomotor behavior that differed from that of worms in the binary chamber assay zone (Fig. 1B-F). Specifically, in the uniform chamber assay zone, worms moved approximately 40% faster (Fig. 1B, F), spent about 20% less time idle (Fig. 1C, F), reversed direction around 20% less frequently (Fig. 1D, F), and turned about 30% more often (Fig. 1E, F) than worms in the binary chamber assay zone. These findings demonstrate that locomotion adjustments are shaped by past sensory experience rather than solely by immediate environmental cues. That worms exhibited different behaviors in identical assay zones highlights the flexible, context-dependent nature of *C. elegans* behavior.

Guanylyl cyclase gene *gcy-18* is required for context-dependent locomotion modulation in chambers.

To identify mechanisms underlying context-dependent locomotion adjustments, we employed a previously reported preference assay that uses microfluidic arenas to assess the ability of worms to select a preferred space [206]. Because worms must adjust their locomotion based on their surroundings to remain within a preferred area, we hypothesized that mutations abolishing this preference would reveal key mechanisms underlying context-dependent adjustments in the uniform and binary chambers. Accordingly, we screened for mutant worms that failed to choose their preferred area, aiming to identify the genetic mechanisms mediating locomotion adjustments in response to different physical structures. Given the significant role of cGMP in neuronal plasticity and behavior modulation, we performed a small-scale genetic screen using loss-of-function mutants for 24 genes encoding guanylyl cyclase (GC), the enzyme responsible for cGMP synthesis. Using the preference assay, we identified two putative null mutants, *gcy-18(gk423024)* and *gcy-12(gk142661),* that failed to display a spatial preference (Supp Fig. 1). We then compared the locomotion properties of *gcy-18* and *gcy-12* mutants in both uniform and binary chambers. Our results showed that *gcy-18* mutants failed to execute context-dependent adjustments, displaying similar locomotion rates in both chamber types.

Specifically, *gcy-18* mutants exhibited a significantly reduced ∆speed (3 ± 5%) compared to wild type (35 ± 7%, p<0.001, Fig. 2A). In contrast, *gcy-12* mutant worms exhibited normal speed adjustment, with ∆speed values comparable to wild type (Fig. 2A). This indicates that *gcy-12* is not required for the modulation of locomotion speed. In comparison, the failure of *gcy-18* mutants to adjust their speed suggests that *gcy-18* plays a critical role in context-dependent behavioral adjustment. Together, these results demonstrate that among the guanylyl cyclases, *gcy-18* is essential for the context-dependent modulation of locomotion.

To confirm that the defects observed in *gcy-18* mutants were not due to background artifacts, we introduced a single-copy transgene that drives *gcy-18* cDNA expression under the control of a *gcy-18* promoter into *gcy-18(gk423024)* mutants to test whether reintroducing a functional copy of *gcy-18* could rescue the behavioral defects. Indeed, expression of the transgene restored locomotion adjustments (∆speed: 20 ± 6%) to levels closer to those observed in wild type animals (35 ± 7%, p = 0.4, Fig. 2B), confirming the crucial role of *gcy-18* in mediating these adjustments based on the experience of exploring physical environments. The identification of *gcy-18* was particularly intriguing, as its expression is restricted to a single pair of neurons, the thermosensory AFDs. Given that our assay chambers lacked thermal gradients, this finding suggests a novel role for *gcy-18* and AFD neurons in mediating locomotion adjustments in response to the exploration of surrounding physical structures.

To further investigate the role of AFD-specific guanylyl cyclases in context-dependent locomotion modulation, we examined *gcy-8*, which is essential for generating thermally evoked Ca^{2+} responses in AFD [160,161]. In thermal responses, disrupting *gcy-8* alone is sufficient to impair AFD function, whereas disruption of *gcy-18* alone does not [161,162], indicating the critical role of GCY-8 in AFD thermosensation. If *gcy-18* influenced behavioral adjustment in microfluidic chambers solely through its role in thermosensation, we would expect that a loss-of-function mutation in *gcy-8* would have a similar or even more disruptive impact on locomotion

modulation. However, *gcy-8* mutant worms exhibit little change in their ability to adjust locomotion speed (Δspeed: 29 ± 7%), with Δspeed values similar to that observed in wild type worms ($p = 0.96$, Fig. 2C). These results indicate that while *gcy-8* is critical for thermosensation, its mutation does not affect context-dependent locomotion modulation, suggesting that independent cGMP signaling mechanisms govern AFD neuronal activity in thermosensation and context-dependent behavior in microfluidic chambers (Fig. 2D).

Distinct CNG channels mediate context-dependent locomotion modulation and thermosensation.

The role of guanylyl cyclase in cGMP synthesis prompted us to investigate whether cyclic nucleotide-gated (CNG) channels are involved in context-dependent locomotion modulation. CNG channels, composed of α and β subunits, open upon binding cyclic nucleotides (*e.g.*, cGMP and cAMP), allowing Ca^{2+} influx [123,207–209] to control signal transduction and neural plasticity across species [210–217] (Fig. 3A). In *C. elegans*, mutations in either the *tax-4* or *tax-2* genes, which encode the α and β subunits of CNG channels respectively, impair thermotaxis behavior [156,218] and disrupt AFD neuron responses to temperature changes [118,219]. To determine their role in context-dependent locomotion modulation, we examined *tax-4(p678)* and *tax-2(p694)* mutant worms using our microfluidic chamber assay.

We found that *tax-2* is essential for context-dependent locomotion modulation, as *tax-2* mutants did not exhibit differences in speed between chamber types (Fig. 3B, C). In contrast, *tax-4* mutants displayed an enhanced locomotion adjustment compared to wild type worms ($p<0.01$; Fig. 3C)—a striking difference from the lack of speed changes seen in *tax-2* mutants. Furthermore, *tax-4* mutants exhibited significantly increased basal locomotion rates in both chambers (Fig 3B), suggesting that alterations in basal locomotion do not interfere with experience-dependent movement adjustments.

Consistent with the idea that basal and experience-dependent locomotion are regulated independently, we found that wild type worms cultivated at either 23ºC or 17ºC and then transferred to 20ºC during chamber exploration exhibited significantly changes in basal locomotion speed but had little corresponding effect on the extent of context-dependent modulation (Supp Fig. 2A). Specifically, temperature shifts were positively correlated with basal locomotion rates (binary chambers: $r = 0.52$, $p < 0.0001$; uniform chambers: $r = 0.48$, $p < 0.0001$; Suppl. Fig. 2B), yet no significant correlation was observed between temperature shifts and Δspeed ($r = -0.03$, $p = 0.22$; Suppl. Fig. 2C), reinforcing the idea that these two processes operate independently.

Together, these observations indicate that TAX-2 is critical for integrating prior experience into locomotion modulation, while TAX-4 primarily regulates basal locomotion. Despite both pathways engaging cGMP signaling, their functional independence suggests distinct regulatory mechanisms for basal locomotion and context-dependent adjustments.

Given that four other genes (*cng-1, cng-2, cng-3* and *cng-4*) are predicted to encode CNG channels in *C. elegans,* we investigated their role in locomotion modulation in microfluidic chambers [163]. Due to abnormal development in *cng-1* mutants, we were unable to assess their locomotion; however, we were able to evaluate mutants of the other three genes. We found that *cng-3(gk541751)* mutant worms failed to adjust locomotion speed in the microfluidic chambers, while *cng-2(tm4267)* and *cng-4(gk195496)* mutants exhibited normal modulation comparable to wild type worms (Fig. 3D). The identification of CNG-3 was particularly intriguing, as recent studies suggest that it mediates changes in AFD neuronal activity based on prior temperature experiences [220,221], indicating that CNG-3 contributes to neural plasticity rather than direct stimulus detection. Our results further support this view by showing that CNG-3 plays a broader role in locomotion modulation in environments lacking thermal gradients, which suggests its involvement in neuronal plasticity beyond the thermosensory modality.

Next, we determined whether AFD is the site where CNG-3 functions in context-dependent locomotion modulation. To do so, we introduced single-copy transgenes expressing *cng-3* cDNA under the control of two AFD-specific promoters, *srtx-1Bp* and *gcy-18p*, into *cng-3(gk541751)* mutant worms. Regardless of the promoter used, AFD-specific expression of *cng-3* restored the locomotion speed modulation (∆speed: 47 ± 8% for *srtx-1Bp*::*cng-3* and 40 ± 6% for *gcy-18p::cng-3*) to levels similar to wild type (Fig 3E). These results demonstrate that AFD neurons are the site of action for CNG-3 in mediating context-dependent locomotion modulation. Overall, our findings elucidate an unexplored role for AFD beyond thermosensation, revealing that components of a cGMP signaling pathway enable AFD to modulate behavior in an experience-dependent manner.

AFD sensory microvilli are dispensable for locomotion modulation, but AFD neurons are required.

The finding that cGMP signaling genes (such as *gcy-18* and *cng-3*) in AFD neurons modulate context-dependent locomotion prompted us to investigate whether the AFD thermosensory apparatus, crucial for temperature detection [203], is also necessary for locomotion modulation in our microfluidic assay. Previous studies have shown that the sensory microvilli at the tip of the AFD dendrite (Fig. 4A) are essential for temperature sensing and thermotaxis [203,205]. For example, mutations in the *kcc-3* gene, which encodes a K^+/Cl^- cotransporter in the amphid sheath glia, disrupt microvilli formation and significantly impair thermotaxis towards their cultivation temperature [204]. Despite these thermotaxis defects, we found that context-dependent locomotion modulation remained unchanged in *kcc-3(ok228)* mutant worms (∆speed: 33 ± 4%) compared to wild type (42 ± 7%; p = 0.28; Fig. 4B, C). Interestingly, *kcc-3* mutant worms displayed elevated basal locomotion rates in both chamber types (Fig. 4B), a phenomenon also observed in *tax-4* mutants. This further supports our earlier finding that alterations in basal locomotion do not correlate with context-dependent locomotion modulation (Suppl. Fig. 2B, C).

Together, these findings suggest that the role of AFD neurons in modulating context-dependent locomotion is distinct from their thermosensory functions and differs from the mechanisms controlling basal locomotion.

To further confirm that the sensory endings of AFD are not required for context-dependent locomotion modulation, we examined *ttx-1(767)* mutant worms, which completely lack microvilli and exhibit more severe thermotaxis defects than those seen in *kcc-3* mutants [205]. Consistent with the notion that AFD has separate roles in context-dependent locomotion and thermosensation, *ttx-1* mutants still display locomotion modulation in microfluidic chambers. However, the extent of modulation in these mutants was slightly reduced and more variable (∆speed: 29 ± 10%) compared to wild type animals (42 ± 7%, p = 0.29, Fig. 4D). It is worth noting that *ttx-1* mutations disrupt the expression of multiple AFD genes, including those involved in cGMP signaling [222], which may explain the increased variability in locomotion modulation. Nonetheless, despite completely lacking the AFD sensory apparatus, the ability of *ttx-1* mutant worms to adjust their locomotion speed further demonstrates that AFD neurons modulate context-dependent locomotion via mechanisms distinct from those used in thermosensation.

Surprised to find that the AFD sensory apparatus is dispensable for locomotion modulation, we next examined worms with genetically ablated AFD neurons. To achieve targeted AFD ablation, we used a published strain with an integrated transgene expressing caspase protein under the AFD-specific promoter *gcy-8p* [223]. AFD-ablated worms lost the ability to adjust locomotion rates in microfluidic chambers, as indicated by a significant reduction in ∆speed (wild type: 42 ± 7%, AFD ablated: -2 ± 7%, p <0.001; Fig. 4E). These results demonstrate that although the sensory endings of AFD are not required for locomotion adjustment, the AFD neuron itself is essential.

Context-dependent locomotion modulation requires the MEC-10 mechanosensory channel subunit.

We investigated whether mechanosensation is necessary for worms to perform context-dependent locomotion adjustments in microfluidic chambers. This was prompted by previous findings showing that touch sensation is critical for *C. elegans* to select their preferred space through interactions with physical structures [206]. The *mec-10* gene encodes an amiloride-sensitive Na^+ channel protein required for sensing body touch. Mutations in *mec-10* disrupt mechanotransduction channels in the worms touch-receptor neurons (TRNs), impairing touch sensitivity [224,225]. We found that *mec-10(tm1552)* mutants failed to display locomotion adjustment (Δspeed: $5 \pm 4\%$, Fig. 5B). These results show that *mec-10* is crucial for context-dependent locomotion modulation, supporting the hypothesis that locomotion is modulated depending on the worm's tactile experience.

AIB interneurons connecting TRNs and AFD are crucial for tactile-dependent behavioral plasticity.

Touch receptor neurons (TRNs) do not directly synapse onto AFD, suggesting that additional neurons are required to bridge them for tactile-dependent locomotion modulation. Examination of the *C. elegans* connectome revealed that AIB serves as the shortest network pathway connecting TRNs to AFD (Fig. 5A). This positions AIB as a key candidate for relaying signals from TRNs to AFD. If this is correct, we expect AIB to be necessary for tactile-dependent modulation. To examine this, we quantified the locomotion of AIB-ablated worms in microfluidic chambers. For this, we employed a previously published strain expressing caspase under an AIB-specific promoter (*npr-9p*), resulting in ablation of the AIB neuron pair [226]. We found AIB-ablated worms failed to adjust their speed in chambers, showing a significant reduction in Δspeed ($-1 \pm 5\%$) compared to wild type ($32 \pm 8\%$, $p < 0.01$, Fig. 5C). These results demonstrate that the AIB interneurons are crucial for tactile-dependent locomotion modulation.

Mutations in *innexin* genes disrupt tactile-dependent modulation.

Electrical synapses between AIB and AFD in *C. elegans* are formed by gap junction proteins called innexins [227]. If these AIB–AFD connections are important for tactile-dependent modulation, then mutations in innexin genes should impair locomotion adjustments in microfluidic chambers. To examine this hypothesis, we investigated loss-of-function mutants of various innexin genes. Because *C. elegans* has 25 innexin genes [228,229], we focused on those known to be expressed in AFD and AIB [230–232], identifying three genes (*inx-4, inx-10* and *inx-19)* expressed in AFD*,* one gene (*inx-1)* in AIB, and one gene (*inx-7*) in both*.* We found that *inx-10* mutants exhibited a partial but significant decrease in locomotion adjustments (∆speed: 15 ± 3%) compared to wild type worms (45 ± 7%, p <0.001, Fig. 6A). Similarly, mutations in *inx-1, inx-4, and inx-7* all lowered ∆speed values, although these differences were not statistically significant relative to wild type worms (Fig. 6A). Furthermore, we found that *inx-7; inx-10* double mutants exhibit a lower ∆speed compared to *inx-7* or *inx-10* single mutants (Fig. 6B). These findings suggest that disrupting innexins in AFD and AIB impair tactile-dependent locomotion modulation.

Re-establishing AIB-AFD electrical synapses restores tactile-dependent locomotion modulation in *inx-7; inx-10* double mutants.

If the tactile-dependent modulation defects in innexin mutants result from disrupted AIB-AFD electrical synapses, then reintroducing a synthetic gap junction specifically between these neurons should rescue locomotion modulation. To test this, we employed a recently developed approach that introduces engineered electrical synapses by expressing the mammalian gap junction protein Cx36 in *C. elegans neurons*. Several studies have shown that engineered Cx36 synapses in *C. elegans* facilitate electrical coupling between neurons, support behavioral

modulation, and circumvent chemosensory impairments in damaged neural circuits [233–236]. Based on these findings, we introduced Cx36 into AFD and AIB neurons in *inx-7; inx-10* double mutants (Fig. 6C). Remarkably, expression of Cx36 in both AFD and AIB significantly improved locomotion adjustments in *inx-7; inx-10* mutants, increasing ∆speed from 15 ± 3% to 31 ± 8% ($p < 0.05$, Fig. 6D). These findings demonstrate that engineered electrical synapses between AFD and AIB can restore tactile-dependent locomotion modulation.

3.4 Discussion

Our study reveals that the thermosensory AFD neuron plays an unexpected role in coupling tactile experiences to locomotion modulation in *C. elegans*. We show that: 1) *C. elegans* exhibit distinct locomotion patterns in identical environments, influenced by prior tactile experiences; 2) tactile-dependent behavior modulation requires the AFD neuron but not its thermosensory dendritic endings; 3) thermotaxis and tactile-dependent locomotion modulation rely on distinct sets of cGMP signaling proteins and CNG channel subunits; 4) the AIB interneuron, which connects AFD to mechanosensory circuits, is essential for tactile modulation; and 5) electrical synapses between AFD and AIB are critical for this process. Below, we discuss the implications of these findings.

A thermosensation-independent role for AFD in tactile-dependent behavior modulation.

Our findings highlight the remarkable versatility of sensory neurons in shaping behaviors beyond their primary functions. This supports the emerging view that sensory neurons have multifaceted roles in circuit modulation, extending well beyond canonical sensory activities. Such complexity is encoded at multiple levels within neural circuits. In *C. elegans*, for instance, the ASH neuron drives avoidance responses to various noxious stimuli, including hyperosmolarity, nose touch, and volatile repellents, demonstrating polymodal functionality [237–

[240]. Similarly, the olfactory receptor neuron AWC exhibits stochastic responses to temperature, introducing variability that enhances the flexibility and reliability of thermotactic behavior [241–243]. In contrast to these examples, the role of AFD in tactile-dependent plasticity does not rely on its sensory endings but is mediated through synaptic connectivity. This reveals an unexpected mechanism by which AFD influences context-dependent behavioral plasticity via network-level interactions rather than direct sensory activity.

Distinct cGMP signaling pathways in AFD mediate sensation and plasticity.

Our data indicates that the CNG channel α subunits TAX-4 and CNG-3 play distinct roles in AFD function. Previous work has shown that TAX-4 is essential for AFD to detect temperature and generate thermally evoked responses [118], whereas CNG-3, although dispensable for temperature sensing, is crucial for tactile-dependent locomotion modulation. Supporting a broader role in sensory plasticity, CNG-3 is required in AWC neurons for short-term odor adaptation [244] and in AFD for adjusting temperature thresholds based on prior thermal experiences [220]. Our demonstration of the essential role of CNG-3 in tactile-dependent locomotion modulation further suggests that CNG-3 enables context-dependent behavioral plasticity rather than directly mediating stimulus detection.

CNG channels are heteromeric complexes composed of both α and β subunits. Our data, along with previous work, show that TAX-2, the CNG β subunit in *C. elegans*, is required for both tactile-dependent locomotion modulation and thermotransduction [118]. This finding implies that TAX-2 can partner with different α subunits to support sensory detection and neural plasticity. This functional specialization is reminiscent of photoreceptor neurons, where the composition and ratio of CNG channel subunits determine light sensitivity and enable contrast detection under varying light intensities [245–247].

In addition to the diversity of CNG channels, guanylyl cyclases in AFD also exhibit functional specificity. For example, mutations in *gcy-18* alone significantly disrupt tactile-

dependent locomotion modulation without substantially affecting temperature sensing [161,162], whereas *gcy-8* is essential for generating thermally evoked Ca^{2+} responses [160,161] yet appears dispensable for tactile-dependent plasticity. Together, these results show that AFD uses distinct sets of guanylyl cyclases and CNG channels to execute dual roles—precise temperature sensing and context-dependent behavioral modulation.

Differential regulation of context-dependent plasticity and basal locomotion rates in AFD.

Our results further suggest that the mechanisms controlling basal locomotion and context-dependent behavioral modulation are not only distinct but also operate independently. Temperature fluctuations significantly affect basal locomotion rates, i.e., higher temperatures accelerate speed, while lower temperatures slow it down [248,249]. However, tactile-dependent locomotion modulation in microfluidic chambers remains robust despite these temperature-induced changes in basal movement. This independence is further supported by our observations in *tax-4* and *kcc-3* mutants, which display elevated basal locomotion rates yet still adjust their locomotion in response to tactile experiences. These findings indicate that the processes governing basal locomotion and context-dependent modulation are controlled by separate, non-interfering mechanisms, even though both pathways engage cGMP signaling.

Electrical synapses bridge distinct circuits for behavioral modulation.

AIB-AFD electrical synapses are pivotal for tactile-dependent behavioral plasticity, serving to connect mechanosensory and thermosensory circuits. Our results show that disrupting the electrical connection between AFD and AIB impairs tactile-dependent locomotion modulation, whereas reintroducing engineered synapses restores it. The successful replacement of invertebrate innexins with vertebrate connexins, proteins that perform similar functions despite lacking sequence homology, indicates that the core function of these synapses lies in their connectivity rather than in their specific protein sequences. Previous models have proposed that

AIB-AFD electrical synapses contribute to avoidance behavior by forming part of an FLP-AIB-AFD circuit, with AIB serving as a hub neuron that integrates sensory inputs via chemical synapse with FLP and electrical synapse with AFD [250]. Our experiments using engineered synapses elucidate the functional importance of the AIB-AFD connection in tactile-dependent locomotion modulation. Moreover, the transfer of signaling molecules such as Ca^{2+} and cGMP through electrical synapses likely facilitates the coordination of neuronal activity across distinct networks [251–254]. Although the precise mechanisms require further investigation, our study demonstrates that AIB-AFD electrical synapses are essential for incorporating tactile context into locomotion modulation.

In Summary, our findings reveal an unexpected role for AFD in tactile-dependent behavioral plasticity. They show that specialized molecular mechanisms (including distinct cGMP signaling pathways, diverse guanylyl cyclases, and functional electrical synapse connectivity) enable AFD sensory neurons to perform dual functions: detect stimuli and support context-dependent plasticity. This work expands our understanding of sensory neuron versatility supporting adaptive behavior in animals.

3.5 Methods.

Nematode Strains and Growth – *C. elegans* strains were maintained under standard conditions at 20°C on nematode growth medium (NGM) agar plates [255]. These plates were seeded with *E. coli* OP50 lawns. Strains used in this study are listed in Table 1.

Single Copy Insertion of Transgenes – *Mos1*-mediated transgene insertion methods were used to generate transgenic animals carrying single-copy transgenes [256,257]. Briefly, worms carrying the *ttTi5605 Mos1* site on chromosome II were injected with a mixture of plasmids

encoding the transgene of interest (40 ng/µl) and Cas9 protein plus guide RNA specific to the *Mos1* site (60 ng/µl). To aid in selection of successful insertions, the inserted transgene also included markers *vha-9p::mScarlet* and *rps-0p::hygR*, which confer red fluorescence in the gut and hygromycin resistance, respectively.

Transgenes and Germline Transformation – Strains carrying extrachromosomal arrays were generated by microinjecting various plasmids together with co-injection markers. The co-injection markers included *unc-112p::gfp* (coelomocyte, 30 ng/µl), *rpl-28p::neoR (20* ng/µl), and *Cbr-unc-119 (20* ng/µl). The blank vector pBluescript was used as filler DNA to bring the final total DNA concentration to 100 ng/µl. A detailed list of the plasmids used to create transgenic worms in this study is provided in Table 2.

Fabrication of PDMS Microfluidic Chambers – Microfluidic chambers were designed in AutoCAD (S1. File), and the resulting CAD designs were used to fabricate SU8-Silicon molds with a height of 100 µm using photolithography (FlowJem, NO, Canada). These molds were then used to fabricate microfluidic chambers with various physical features using soft lithography with polydimethyl siloxane (PDMS, Sylgard 184, Dow Corning, MI). Specifically, PDMS prepolymer was poured onto the SU8-silicon molds and cured at 80°C for 2 hours. Once cured, the PDMS layer was carefully lifted away from the silicon wafer, and a 2 mm skin biopsy punch (Acuderm Inc., FL) was used to create inlet and outlet holes. To assemble the device, the PDMS layer was place onto a microscope slide (1 mm thick), and the assembly was treated in a plasma cleaner (PDC-32G, Harrick Scientific, NY) for 1 minute. Distilled water was added to the plasma-treated devices within 5 minutes. Before loading the worms, the devices were thoroughly rinsed with M9 buffer.

Behavior Analysis – Worms were age-synchronized for behavioral assays by transferring 40–50 young adult worms onto an NGM plate seeded with OP50 and allowing them to lay eggs for 2 hours before removal. The plates were then kept at room temperature (20 ± 2°C) for 72 hours, allowing the eggs to develop into young adults. To wash the synchronized adults off the plate, worms were gently transferred into Eppendorf tubes using glass Pasteur pipettes. After allowing the worms to settle at the bottom of the tube (~1 minute), the supernatant was removed and replaced with fresh M9 buffer. This washing step was repeated three times to remove residual OP50. Next, approximately 30–70 worms were loaded into the chamber inlet using a 20µl pipette and allowed to explore the chamber freely for 60 minutes before recording. Videos were captured for 10 seconds at 7.5 frames per second using a Basler acA2440 camera (Basler, PA) equipped with an AF Micro-NIKKOR 60 mm lens (Nikon, NY). Behavioral data were extracted from the videos using WormLab software (MBF Bioscience, VT) through individual worm tracking. Only those worms that were already within the assay area at the start of the recording were analyzed, and any worms that entered later were excluded. Crawling speed was determined by the total distance traveled by a worm during the 10 second video. A turn was defined as a change in crawling direction of 15° or more. Reversals were defined as sudden movement in the opposite direction for at least 5 frames (~0.6 seconds). Worms were considered idle if their absolute velocity (distance traveled between frames) was below 20 µm/s for more than 10 frames (~1.3 seconds).

Statistical Analysis – Behavior measurements were calculated for each individual chamber, and these chamber-specific values were then used to generate the final mean values shown in the bar plots. The sample size was defined as the number (approximately 25) of chambers analyzed per experiment. An unpaired Student's t-test with Bonferroni correction was used to compare average speeds between uniform and binary chambers. To quantify the relative difference between groups, we calculated the percent change for each value in the uniform

chambers relative to the mean of value from the binary chambers. Specifically, we subtracted the mean of the values from the binary chamber from each individual value in the uniform chamber. The result was then divided by the mean of the binary chamber and multiplied by 100 to express the difference as a percentage. For comparisons among multiple strains, we conducted one-way ANOVA followed by a Tukey–Kramer post hoc test on all possible pairwise comparisons. All statistical analyses were performed using R.

3.6 Figures and Tables.

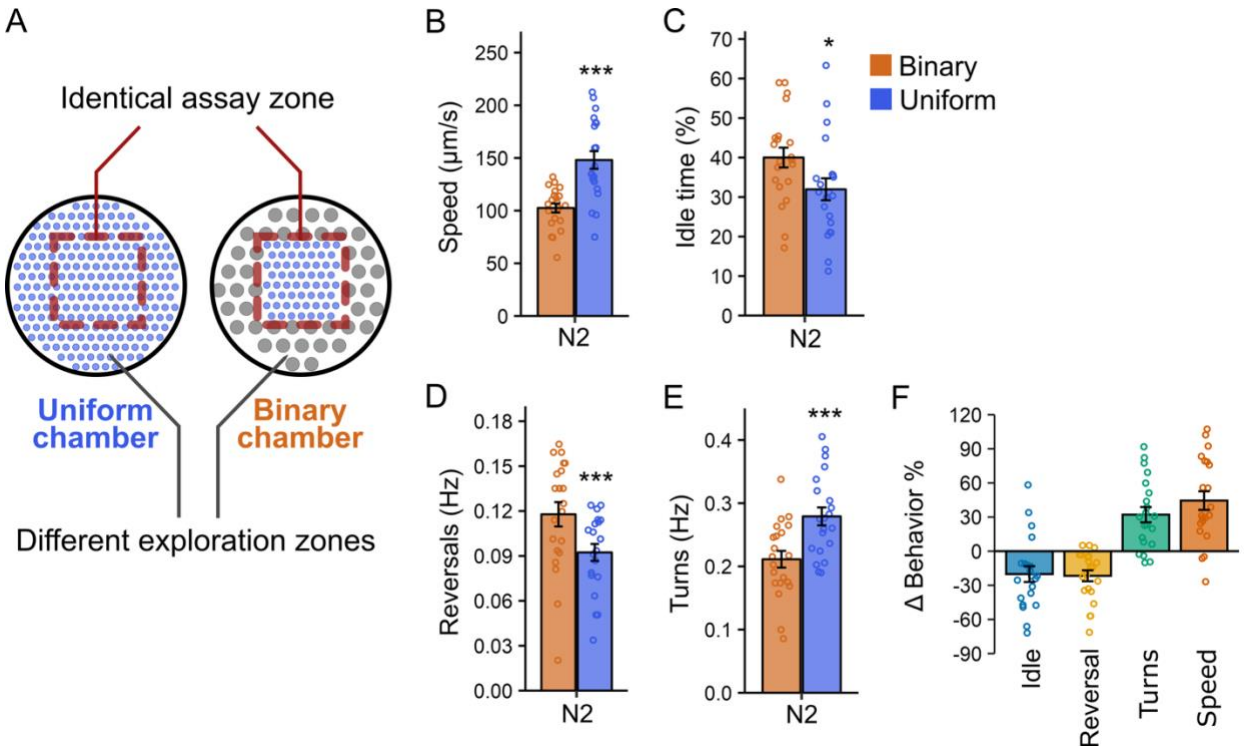


Figure 1. *C. elegans* displays distinct locomotion in identical environments after exploring different surrounding areas. (A) Schematic of two microfluidic chamber designs—uniform and binary—used for behavioral assays. Both chambers feature an identical assay area (red dashed-line boxes) where locomotion was analyzed, while the exploration zones contain distinct PDMS pillar patterns for worm exploration. (B–E) Bar graphs showing locomotion properties of wild type N2 worms in the assay zones of both chamber types after 1 hour of exploration. Data from binary chambers are shown in orange and from uniform chambers in blue. Shown are (B) locomotion speed, (C) percentage of time spent idle, (D) reversal frequency, and (E) turning frequency. Error bars represent standard error of the mean (SEM); each data point represents the mean behavior value of worms within a chamber. (F) Percent changes in locomotion properties, calculated from data in panels B–E and normalized to the mean value obtained from binary chambers (see Methods), are presented as mean ± SEM. Statistical significance was determined using an unpaired Student's t-test (***: $p < 0.001$, *: $p < 0.05$).

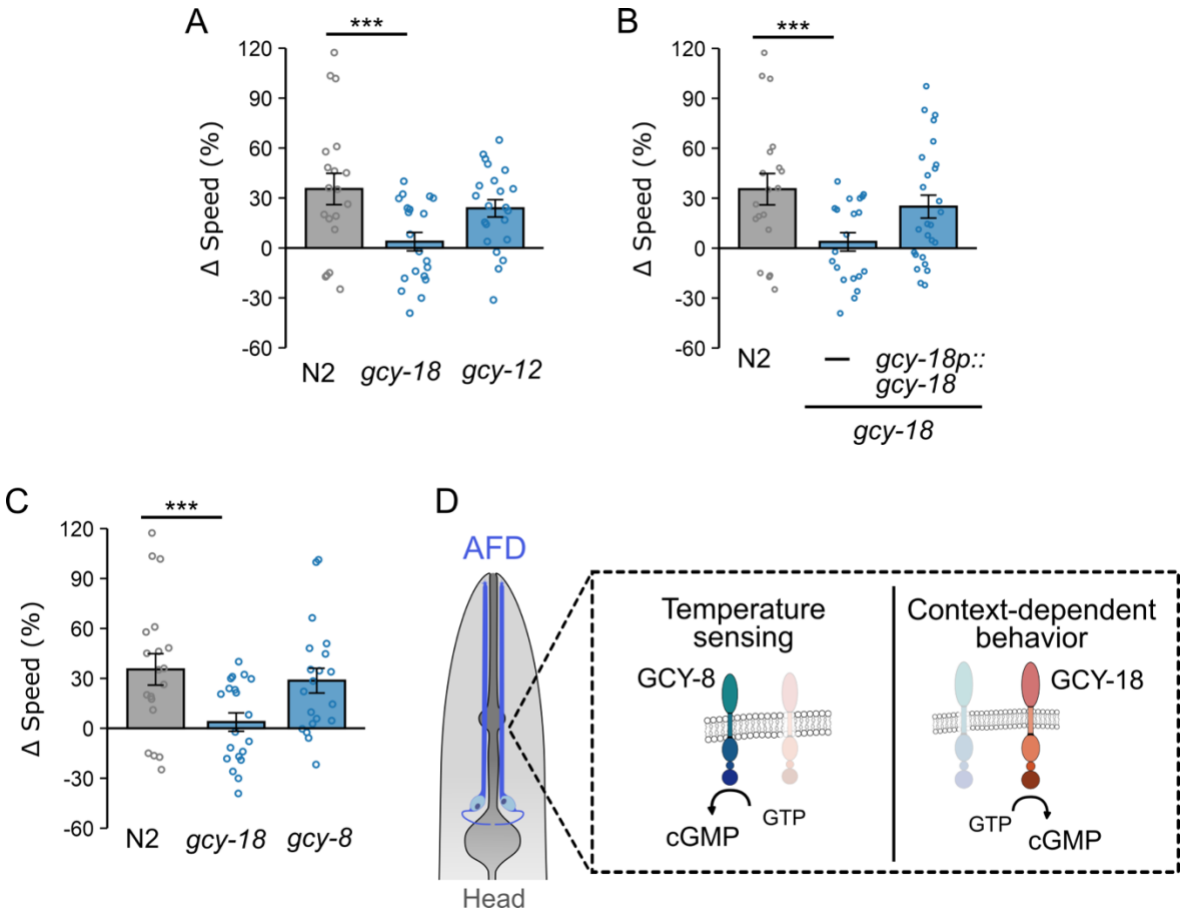


Figure 2. Guanylate cyclase gene *gcy-18* functions in AFD to mediate context-dependent locomotion modulation. (A) Speed differences (Δspeed) between uniform and binary chambers of wild type N2, *gcy-18* and *gcy-12* mutant worms. ΔSpeed was calculated as the percent change in locomotion rates in uniform chambers, relative to the mean of values obtained in binary chambers (see Methods). Mutant worms lacking *gcy-18* failed to demonstrate context-dependent adjustments in locomotion speed, as indicated by a low Δspeed value, whereas *gcy-12* mutants behaved similarly to wild type worms. (B) A single-copy transgene expressing *gcy-18* cDNA under the control of the *gcy-8* promoter in AFD restores context-dependent locomotion modulation. (C) Disruption of *gcy-8* does not impair context-dependent locomotion modulation. (D) Schematic illustrating the distinct roles of guanylate cyclase genes in AFD sensory neurons: *gcy-8* is required for thermosensation but not for context-dependent locomotion modulation, whereas *gcy-18* is essential for context-dependent locomotion modulation and plays only a moderate role in thermosensation. Data are presented as mean ± SEM. Each data point represents the mean behavior of worms within a single chamber. Statistical significance was determined using one-way ANOVA followed by a Tukey–Kramer post hoc test (***: $p < 0.001$).

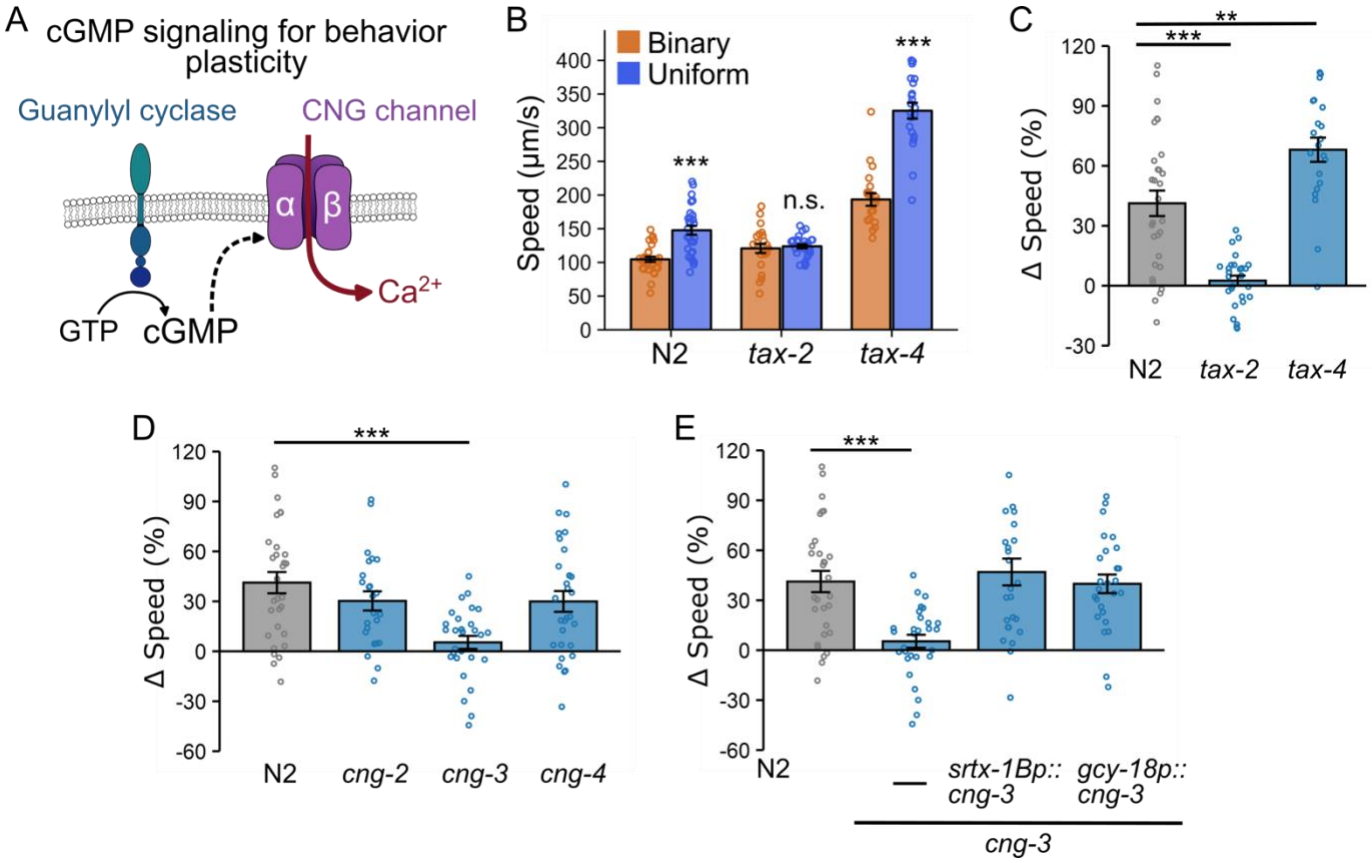


Figure 3. Cyclic nucleotide-gated (CNG) channel subunits TAX-2 and CNG-3 are required for locomotion modulation, but TAX-4 is not. (A) Schematic representation of CNG channel function. CNG channels are activated by cyclic nucleotides (such as cGMP) and mediate Ca^{2+} influx, thereby influencing neuronal activity and animal behavior. (B) Locomotion speed (µm/s) of N2, *tax-2*, and *tax-4* mutant worms in binary (orange) and uniform (blue) chambers. The *tax-2* mutants exhibit identical locomotion rates in both chamber types, whereas worms lacking *tax-4* display accelerated basal locomotion while still preserving context-dependent locomotion modulation. (C) Speed differences (Δspeed) for N2, *tax-2* mutant, and *tax-4* mutant worms. The *tax-2* mutants failed to modulate locomotion rates in a context-dependent manner, whereas *tax-4* mutations enhanced modulation. (D) Assessment of *cng-2*, *cng-3*, and *cng-4* roles in locomotion adjustments. The *cng-3* mutation abolishes locomotion modulation. (E) Single-copy transgenes expressing *cng-3* cDNA under two different AFD-specific promoters restore context-dependent locomotion adjustments. Data are presented as mean ± SEM. Each data point represents the mean behavior of worms within a single chamber. Statistical significance was determined using an unpaired Student's t-test to compare speeds between uniform and binary chambers (panel B) and one-way ANOVA followed by a Tukey–Kramer post hoc test to compare Δspeed across strains (panels C-E) (*: $p < 0.05$; **: $p < 0.01$, and ***: $p < 0.001$).

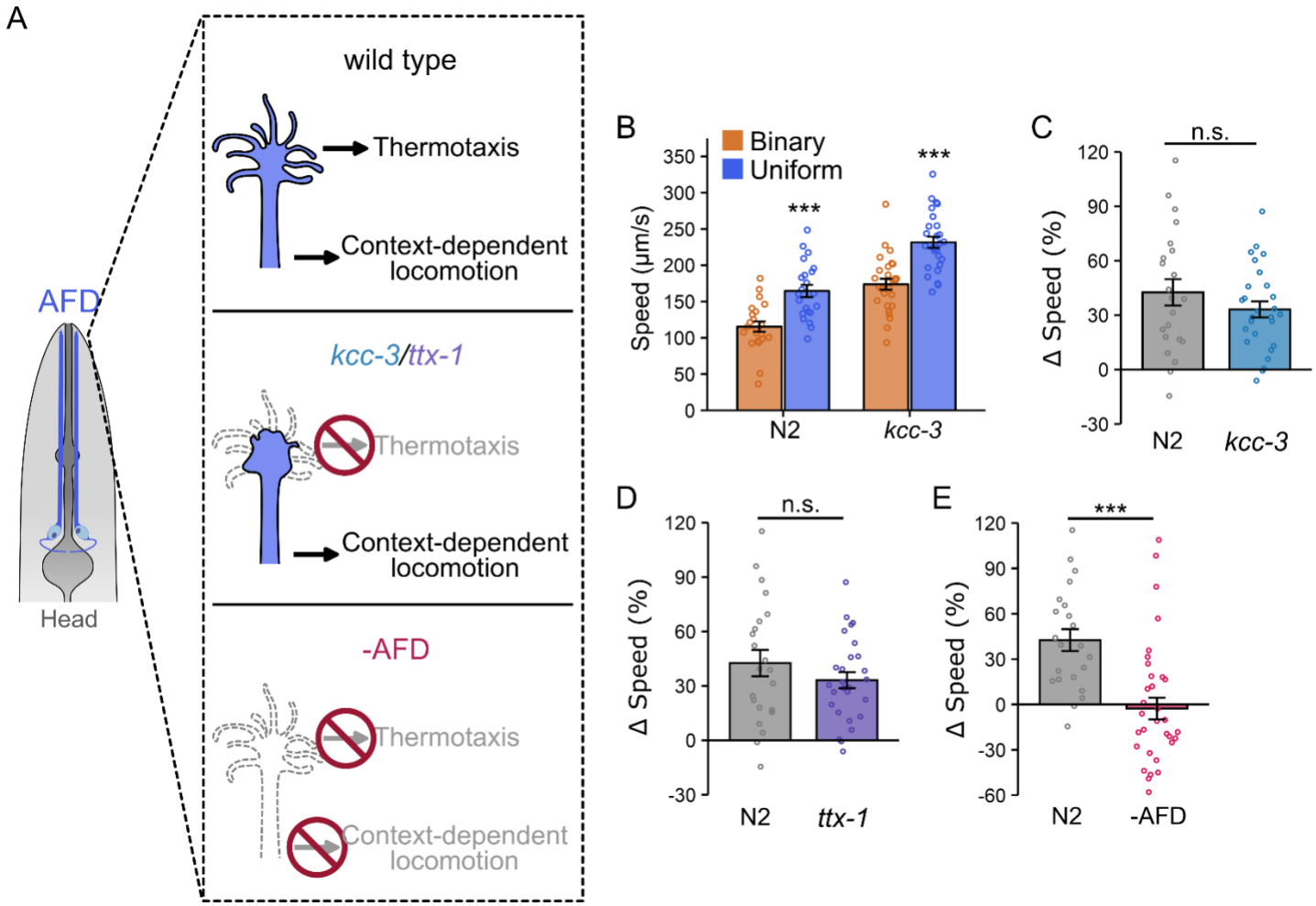


Figure 4. AFD, but not its sensory endings, is required for context-dependent locomotion modulation. (A) Schematic representation of AFD function in temperature sensing and locomotion modulation. AFD sensory endings are essential for thermosensation but dispensable for context-dependent locomotion modulation. (B) Locomotion speed (μm/s) of wild type N2 and *kcc-3* mutant worms in the binary (orange) and uniform (blue) chambers. The *kcc-3* mutants preserve context-dependent locomotion modulation while exhibiting increased basal locomotion rates. (C) Speed differences (Δspeed) for N2 and *kcc-3* mutant worms, and (D) for N2 and *ttx-1* mutant worms, respectively. Context-dependent locomotion modulation remains in *kcc-3* and *ttx-1* mutant worms, although they abolish the AFD thermosensory function. (E) Ablation of AFD eliminates the context-dependent locomotion modulation. Data are presented as mean ± SEM. Each data point represents the mean behavior of worms within a single chamber. Statistical significance was determined using an unpaired Student's t-text (n.s. indicates not significant, and ***: $p < 0.001$).

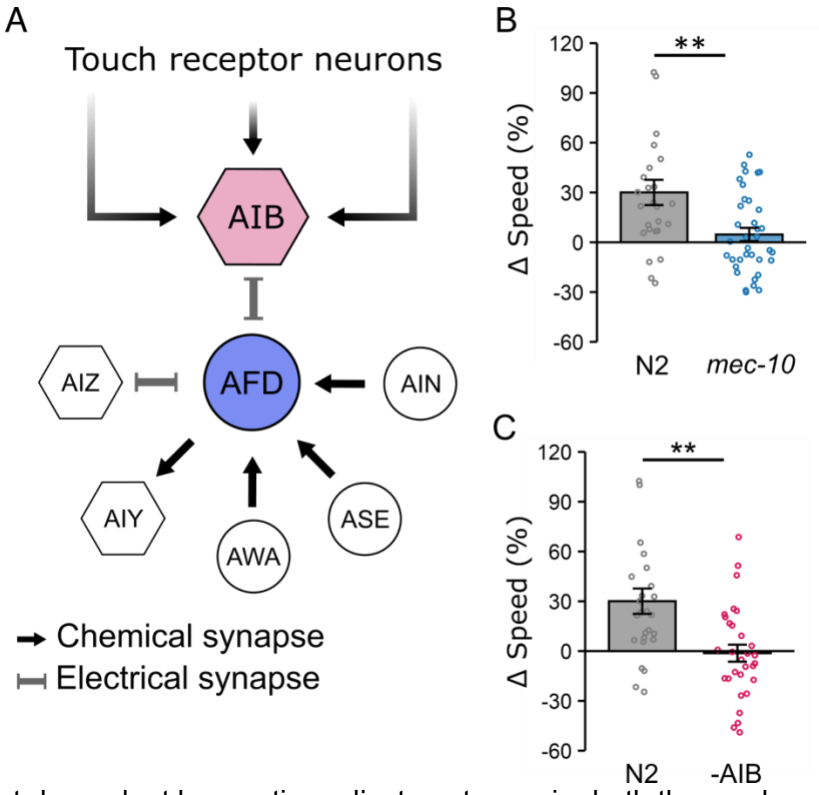


Figure 5. Context-dependent locomotion adjustments require both the mechanosensory channel subunit MEC-10 and the AIB interneurons that connect AFD to touch circuits. (A) Schematic representation of neurons synapsing with AFD. AIB provides the shortest pathway linking AFD to touch receptor neurons. (B, C) Speed differences (Δspeed) for N2, *mec-10* mutants, and AIB-ablated (-AIB) worms. *Both mec-10* mutants (B) and AIB-ablated worms (C) fail to modulate locomotion in microfluidic chambers. Data are presented as mean ± SEM. Each data point represents the mean behavior of worms within a single chamber. Statistical significance was determined using one-way ANOVA followed by a Tukey–Kramer post hoc test (**: $p < 0.01$).

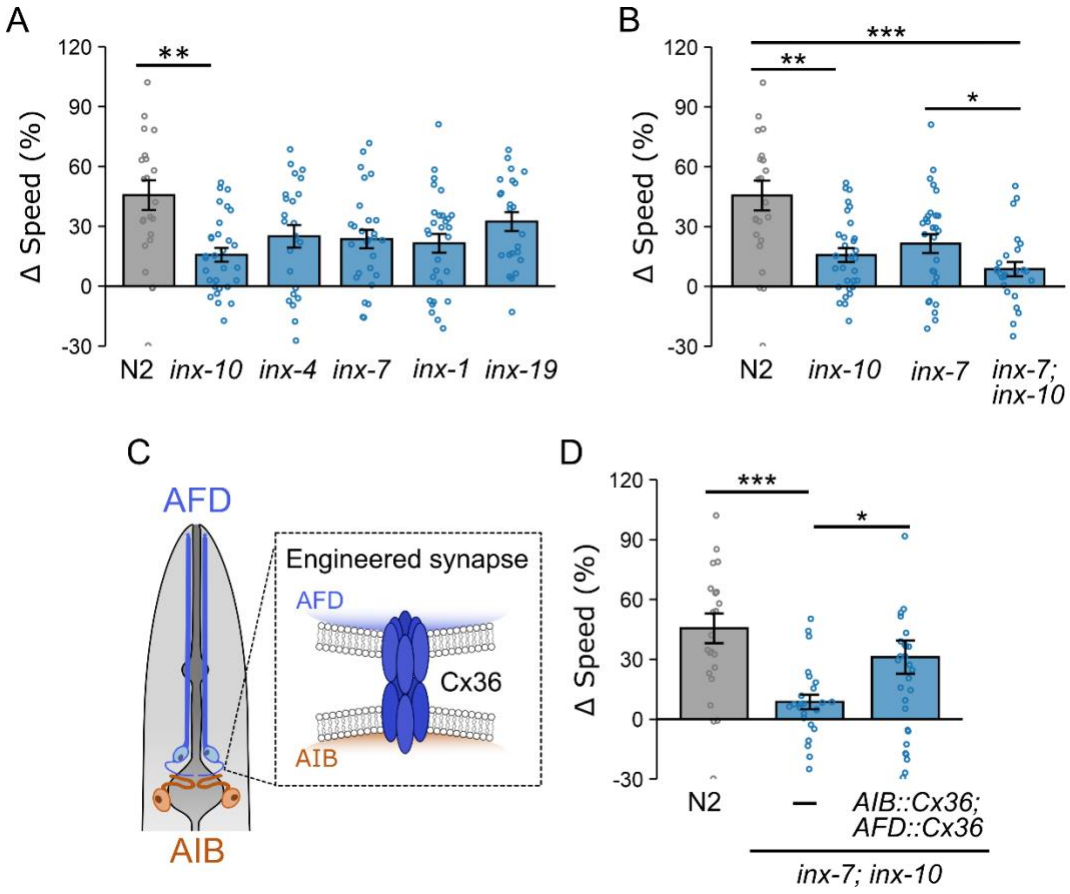
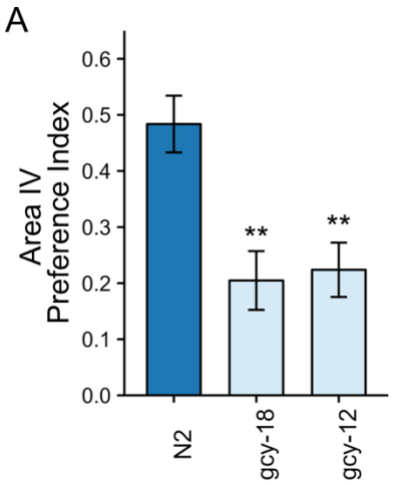
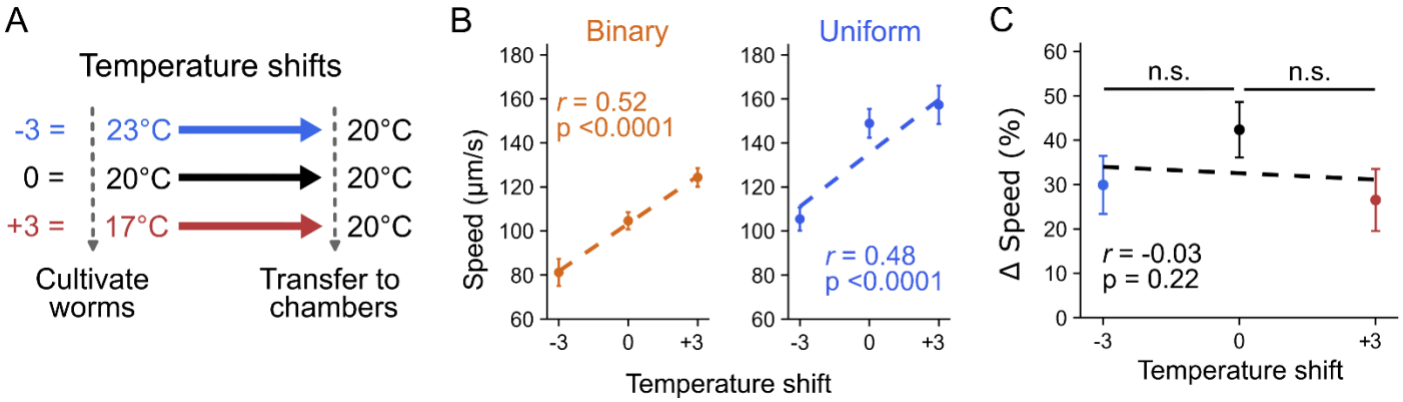


Figure 6. Tactile-dependent locomotion modulation is disrupted in mutant worms lacking gap junction genes and is restored by engineered Cx36 electrical synapses linking AFD and AIB. (A) Speed differences (Δspeed) for *inx-1, 4, 7, 10, 19* mutant worms. (B) Speed differences for *inx-7; inx-10* double mutants. (C) Schematic illustrating engineered electrical synapses formed by Cx36 when expressed in adjacent neurons. (D) Tactile-dependent locomotion modulation is restored in *inx-7; inx-10* double mutants by expressing Cx36 in AFD and AIB neurons. Data are presented as mean ± SEM. Each data point represents the mean behavior of worms within a single chamber. Statistical significance was determined using one-way ANOVA followed by a Tukey–Kramer post hoc test (*: $p < 0.05$; **: $p < 0.01$, and ***: $p < 0.001$).



Suppl. Figure 1. Worms lacking either *gcy-18* or *gcy-12* failed to identify their preferred area in microfluidic chambers. Preference index values of wild type N2 worms, *gcy-18* mutants, and *gcy-12* mutants were determined as described by Han *et al.* [206]. Wild type worms exhibit a strong preference for a specific zone (zone IV, as defined by Han *et al*. [206]), whereas both *gcy-18* and *gcy-12* mutants show significantly reduced preference compared to wild type worms. Bars represent mean ± SEM. Statistical comparisons were performed using one-way ANOVA followed by a Dunnett's test for multiple comparisons (**p < 0.01).



Suppl. figure 2. Temperature shifts do not affect tactile-dependent modulation. (A) Schematic of experimental design: Worms were reared at three different temperatures (17°C, 20°C, and 23°C) and then transferred to 20°C for 1 hour during the behavioral assay. (B) Locomotion speed of wild type N2 worms in the binary (orange) and uniform (blue) chambers after experiencing temperature shifts. Linear regression analysis reveals a significant positive correlation between rearing temperature and locomotion speed in both chambers. (C) Differences in speed (Δspeed) between worms experiencing different temperature shifts. Linear regression significance test shows no significant relationship between Δspeed and temperature shift. Points represent mean ± SEM.

Table 1. List of *C. elegans* strains used in this study

Strain	Genotype	Citation
PR694	*tax-2(p694) I*	258
PR678	*tax-4(p678) III*	218
FX31482	*cng-2(tm4267) IV*	259
VC40261	*cng-3(gk541751) IV*	260
VC20290	*cng-4(gk195496) IV*	260
IK800	*gcy-8(oy44) IV.*	162
VC30137	*gcy-18(gk423024) IV*	260
VC20451	*gcy-12(gk142661) II*	260
BJH3481	*gcy-18(gk423024) IV, pekSi609[gcy-18p::gcy-18(cDNA)::sl2::mNeonGreen::unc-54 3'utr, vha-6p::mScarlet, hygR(+)] ttTi5605 II*	This study
BJH3527	*pekSi655[srtx-1Bp::cng-3(cDNA)::sl2::mNeonGreen::unc-54 3'utr, vha-9p::mScarlet, hygR(+)] ttTi5605 II; cng-3(gk541751) IV*	This study
BJH3537	*tax-2(p694) I; pekSi665[srtx-1Bp::tax2(cDNA)::sl2::mNeonGreen ::unc-54 3'utr, vha-9p::mScarlet, hygR(+)] ttTi5605 II*	This study
BJH3539	*pekSi667 [gcy-18p::cng-3(cDNA)::sl2::mNeonGreen::unc-54* 3'utr, *vha-9p::mScarlet, hygR(+)] ttTi5605 II; cng-3(gk541751) IV*	This study
GN112	*pgIs2[gcy-8p::TU#813, gcy-8p::TU#814, unc-122p::GFP, gcy-8p::mCherry, gcy-8p::gfp, ttx-3p::gfp]*	223
LX1024	*kcc-3(ok228) II*	204
PR767	*ttx-1(p767) V*	205
JN578	*peIs578 [npr-9p::casp1 + npr-9p::venus + unc-122p::mCherry]* (genetically ablated AIB)	226
ZB2551	*mec-10(tm1552) X*	224
CB1611	*mec-4(e1611) X*	261
FG927	*inx-1(tm6662*) X.	262
FG0614	*inx-4 (ok2373) V*	232
RB1792	*inx-7(ok2319)* IV	262
RB2051	*inx-10(ok2714) V*	262
FX01896	*inx-19(tm1896) I*	150
BJH2919	*inx-7(ok2319) IV; inx-10(ok2714) V*	This study
BJH1162	*pekEx302 [srtx-1Bp::cx36*::sl2*::mNeonGreen, inx-1p::cx36 ::*sl2*::mKate, Cbr-unc-119(+), unc-122p::*GFP*, neoR(+)]; unc-119(ed3) III; inx-7(ok2319) IV; inx-10(ok2714) V*	This study

Table 2. List of plasmids used to create transgenic *C. elegans* strains

Name	Description	Notes
BJP-MR16	*gcy-18p::gcy-18(mini-gene)::sl2::mNeonGreen::rab-3 3'utr; vha-9p::mScarlet::tbb-2 3'utr; rps-0p::hygR*	The *gcy-18* mini-gene includes all exons and introns 11, 12, and 13.
BJP-MR65	*srtx-1Bp::cng-3(cDNA)::sl2::mNeonGreen::rab-3 3'utr; vha-9p::mScarlet::tbb-2 3'utr; rps-0p::hygR*	
BJP-MR72	*srtx-1Bp::tax2(cDNA)::sl2::mNeonGreen::rab-3 3'utr; vha-9p::mScarlet::tbb-2 3'utr; rps-0p::hygR*	
BJP-MR93	*gcy-18p::cng-3(cDNA)::sl2::mNeonGreen::rab-3 3'utr; vha-9p::mScarlet::tbb-2 3'utr; rps-0p::hygR*	
BJP-MR96	*inx-1p::cx36::sl2::mKate::rab-3 3'utr*	
BJP-MR97	*srtx-1bp::cx36::sl2::mKate::rab-3 3'utr*	

4. Study limitations and Future Directions.

This study identifies key genes of a cGMP signaling pathway involved in tactile-dependent behavior modulation and raises new questions about their roles in circuit function. In particular, the ability of AFD to mediate tactile modulation without its canonical sensory apparatus suggests novel mechanisms of signal integration and transmission for tactile-dependent behavior. Below, I outline experiments aimed at clarifying the molecular and circuit-level basis of this modulation.

Our findings reveal that components of the cGMP signaling pathway involved in thermosensation are also required for tactile-dependent behavior modulation, yet the two pathways function independently. This raises the possibility that spatial compartmentalization, either of the signaling components themselves or of their downstream targets, enables these pathways to act independently within the same neuron. For instance, GCY-18, and TAX-2 localize to AFD's dendritic ending, where they mediate thermal responses, suggesting this compartment supports robust cGMP signaling [61,157,263]. However, tactile-dependent modulation remains intact in *kcc-3* and *ttx-1* mutants, which lack properly developed sensory endings [204,205], indicating that these structures are not required for tactile signaling. This suggests that cGMP signaling for tactile-dependent modulation may occur in other neuronal compartments or that, in these mutants, signaling components are mislocalized to alternate sites where they retain function. To test these possibilities, we will generate CRISPR/Cas9-edited strains expressing endogenously tagged versions of *gcy-18*, *tax-2*, and *cng-3*, genes required for tactile modulation. Examining tagged proteins in a wild-type background will reveal whether the dendritic ending is their exclusive site of localization or if other compartments are involved. We will also assess protein localization in *ttx-1* and *kcc-3* mutants to determine how sensory-ending disruption affects their distribution. This approach will clarify where cGMP signaling occurs to support tactile-dependent modulation of locomotion.

The circuit logic underlying tactile-dependent modulation remains an open question, particularly how mechanosensory information reaches AFD or AIB. Our data support a model in which an electrical synapse between these neurons enables tactile-dependent behavioral modulation. However, most mechanosensory neurons do not form direct synapses with AFD or AIB, suggesting that extra-synaptic mechanisms may relay tactile information. One well-characterized form of such signaling in *C. elegans* is neuropeptide release from dense-core vesicles (DCVs). DCVs co-package inactive precursors of neuropeptides with proprotein convertases (PCs), which cleave inactive precursors into active neuropeptides [264,265]. Notably, the PC gene *egl-3* is both required and sufficient in mechanosensory neurons for neuropeptide signaling that drives touch-evoked behavioral adaptation, indicating this gene is likely to be involved in extra-synaptic signaling from mechanosensory neurons [264]. Moreover, Neuropeptides are secreted through calcium-triggered exocytosis of DCVs, a process dependent on *unc-31*, which encodes the calcium-dependent activator protein for secretion. To test whether neuropeptide secretion from mechanosensory neurons is necessary for tactile-dependent modulation, we will use two approaches: (1) cell-specific degradation of UNC-31 using the Auxin-Inducible Degradation (AID) system [266], and (2) conditional knockout of *egl-3* in mechanosensory neurons via a Cre/loxP strategy [267]. If neuropeptide signaling is required, we expect these manipulations to impair the worm's ability to modulate behavior based on tactile context. These experiments will provide a framework to study how extra-synaptic signaling expands the computational capacity of neural circuits beyond what is predicted by synaptic connectivity alone.

In addition to the possibility that extra-synaptic signaling contributes to tactile-dependent modulation, prior studies have identified the multimodal neuron FLP as a strong candidate for mediating this behavior via direct synaptic input to AIB. FLP is required to coordinate behavioral responses to noxious heat along with AFD and AIB, placing it within the same circuit framework

examined in this study [268]. Furthermore, *mec-10* null mutants—lacking mechanotransduction in multiple neurons—show reduced spatial preference in microfluidic environments like those used in our study. This defect is rescued by restoring *mec-10* expression specifically in FLP, indicating that FLP is sufficient to drive tactile-dependent modulation [206]. To test this directly, we will express *mec-10* under the FLP-specific *egl-44* promoter in a *mec-10* null background and assess behavioral rescue. This experiment will determine whether mechanosensory input from FLP alone is sufficient to modulate locomotion in response to tactile context.

Our findings demonstrate that components of a cGMP signaling pathway are essential for behavioral modulation, providing a foundation to investigate how these genes influence neuronal activity. Given that the electrical synapse between AFD and AIB likely facilitates bidirectional flow of molecules, two models could explain how cGMP signaling contributes to circuit modulation: either Ca^{2+} diffuses from AIB into AFD to trigger cGMP signaling, or cGMP produced in AFD diffuses into AIB to modulate its activity. To test these possibilities, we will explore two experimental setups that combine optogenetics with functional imaging using GCaMP and the cGMP reporter FlincG3. To assess whether AIB activity modulates AFD, we will express the red-shifted channel rhodopsin Chrimson in AIB and GCaMP or FlincG3 in AFD. If AIB influences AFD, we expect to observe changes in Ca^{2+} and cGMP levels in AFD following optogenetic stimulation. Conversely, to test whether AFD modulates AIB, we will express Chrimson or the light-activated guanylyl cyclase WincG2 in AFD and monitor Ca^{2+} or cGMP levels in AIB. Follow-up experiments using genetic mutants will further clarify the roles of the identified genes in these modulatory effects. Together, these experiments will reveal how AIB and AFD influence one another to coordinate behavioral output.

The experiments outlined here aim to resolve outstanding questions about the localization and function of cGMP signaling components, the circuit pathways linking

mechanosensory input to behavioral output, and the neuromodulation between AFD and AIB. By integrating genetic, imaging, and optogenetic tools, this work will clarify the molecular logic underlying AFD's dual functions and provide broader insight into how sensory neurons can act as interneurons in different contexts to drive adaptive behavior.

5. Broader Impact.

A central goal in neuroscience is to understand how fixed anatomical wiring gives rise to flexible behaviors. In chapter 3, our finding that AFD neurons modulate behavior based on tactile experience—independently of their thermosensory function—provides new insight into how sensory neurons contribute to circuit dynamics. Rather than acting solely as detectors of specific environmental cues, AFD illustrates how sensory neurons can integrate information across modalities to support context-dependent behavioral modulation. These results underscore the functional versatility of sensory neurons and highlight the importance of circuit connectivity in enabling cross-modal communication within neural networks.

While much emphasis has been placed on chemical synapses as sites of plasticity, our findings identify electrical synapses as key participants in tactile-dependent modulation. The ability to restore behavioral plasticity using engineered gap junctions formed from mammalian genes suggests a conserved structural and functional role for electrical synapses across species. This finding also raises the possibility that plasticity at electrical synapses, through changes in coupling strength, molecular composition, or directionality of signal flow, may contribute to flexible circuit function in ways that remain largely unexplored. More broadly, this supports the view that circuit flexibility arises not only from changes in synaptic strength, but also from rerouting information flow through alternative pathways embedded in the connectome.

Our results also point to a modular organization of conserved intracellular signaling mechanisms. Components of the cGMP pathway, such as guanylyl cyclases and CNG channels, appear to function in distinct combinations to support different aspects of neuronal activity, even within a single cell. This suggests that overlapping signal transduction pathways can coexist and be selectively engaged to coordinate both stimulus detection and behavioral adaptation. Because cGMP signaling is broadly conserved across species, understanding how it is differentially deployed within individual neurons offers a framework for investigating how second messengers contribute to plasticity in more complex nervous systems.

Together, this work advances our understanding of circuit modulation in the nematode *C. elegans*. Importantly, it establishes a framework for mechanistic studies of electrical synapse dynamics and the modular organization of second messenger signaling in behavioral plasticity. By revealing how conserved molecular pathways and synaptic architectures support behavioral flexibility, this work may ultimately inform efforts to model, diagnose, or intervene in neurological disorders where these mechanisms are disrupted. As circuit-level dysfunction is increasingly recognized as a hallmark of brain disease, simple models like *C. elegans* offer a useful entry point for developing new tools to study the neural mechanisms underlying behavioral plasticity in both health and disease.

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