Co-option of neurotransmitter signaling for inter-organismal communication in C. elegans

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Biogenic amine neurotransmitters play a central role in metazoan biology, and both their chemical structures and cognate receptors are evolutionarily conserved. Their primary roles are in cell-to-cell signaling, as biogenic amines are not normally recruited for communication between separate individuals. Here, we show that in the nematode C. elegans, a neurotransmitter-sensing G protein-coupled receptor, TYRA-2, is required for avoidance responses to osas9, an ascaroside pheromone that incorporates the neurotransmitter, octopamine. Neuronal ablation, cell-specific genetic rescue, and calcium imaging show that tyra-2 expression in the nociceptive neuron, ASH, is necessary and sufficient to induce osas9 avoidance. Ectopic expression in the AWA neuron, which is generally associated with attractive responses, reverses the response to osas9, resulting in attraction instead of avoidance behavior, confirming that TYRA-2 partakes in the sensing of osas9. The TYRA-2/osas9 signaling system represents an inter-organismal communication channel that evolved via co-option of a neurotransmitter and its cognate receptor.
Inter-organismal communication occurs in many forms across the animal kingdom, both within and between species. Chemosensation, both ancient and ubiquitous across all kingdoms of life, underlies social responses mediated by chemical communication. Social chemical communication requires both cell-to-cell and inter-organismal signaling. First, a chemical cue is released into the environment by one organism that is then detected by specific receptors in another organism. Upon sensation, inter-cellular signaling pathways, e.g., neurotransmitter signaling, are activated that ultimately coordinate a social response.

Neurotransmitter monoamines, such as dopamine, serotonin, tyramine, and octopamine, serve diverse functions across kingdoms. The associated signaling pathways often rely on highly regulated biosyntheses, translocation (either by way of diffusion or through active transport), and perception by dedicated chemoreceptors. Many neurotransmitters are perceived via G protein-coupled receptors (GPCRs); in fact, there is a close relationship between GPCR diversification and neurotransmitter synthesis in shaping neuronal systems. Notably, the most common neurotransmitters share similar behavioral functions across phyla. For example, serotonin is commonly involved in regulating food responses. Other neurotransmitters, such as tyramine and octopamine, are only found in trace amounts in invertebrates, and act as adrenergic signaling compound in vertebrates.

The nematode Caenorhabditis elegans offers many advantages for studying social chemical communication and neuronal signaling, namely the animal’s tractability, well-characterized nervous system, and robust social behavioral responses to pheromones. C. elegans secretes a class of small molecules, the ascaroside pheromones, which serve diverse functions in inter-organismal chemical signaling. As a core feature, these molecules include an ascarylose sugar attached to a fatty acid-derived side chain that can be optionally decorated with building blocks from other primary metabolic pathways. Ascaroside production, and thus the profile of relayed chemical messages, is strongly dependent on the animal’s sex, life stage, environment, and physiological state. Depending on their specific chemical structures and concentration, the effects of ascaroside signaling vary from social (e.g., attraction to icas3#) to developmental (e.g., induction of dauer by asc#8; Fig. 1a)12–15. Furthermore, different combinations of these ascarosides can act synergistically to elicit a stronger behavioral response than one ascaroside alone, such as mate attraction to asc#2, asc#3, and asc#4. Several GPCRs have been identified as chemoreceptors of ascaroside pheromones, such as SRX-43 in ASI to promote dwelling behavior, and DAF-37 in ASK regulating hermaphrodite repulsion.

Recently, an ascaroside, named osas#9, that incorporates the neurotransmitter octopamine, was identified. Osas#9 is produced in large quantities specifically by starved L1 larvae, and elicits aversive responses in starved, but not well fed, animals. The dependency on starvation of both its production and elicited response suggests osas#9 relays information on physiological status and unfavorable foraging conditions. However, it is unknown how osas#9 is perceived and drives starvation-dependent behavioral responses. Based on the unusual incorporation of a monoamine neurotransmitter building block in osas#9, we asked whether other components of monoamine signaling pathways have been recruited for inter-organismal signaling via osas#9. Here, we show that TYRA-2, an endogenous trace amine receptor, is required for the perception of osas#9, demonstrating co-option of a neurotransmitter and a neurotransmitter receptor for inter-organismal communication.

Results
Aversive responses to osas#9 require the GPCR TYRA-2. Previous work has shown that production of the ascaroside osas#9 (Fig. 1a) is starkly increased in starved L1 larvae, and elicits avoidance behavior in starved young adult hermaphrodites (Fig. 1b). This starvation-dependent response is reversible: when worms are starved for an hour, and then reintroduced to food for two hours, no avoidance behavior is observed (Supplementary Fig. 1A). In this study, we tested a broader range of conditions, and found that osas#9 elicits avoidance regardless of the sex or the developmental stage of the animal (Fig. 1c), and that osas#9 is active over a broad range of concentrations (µM – µM; Supplementary Fig. 1B). 1 µM osas#9 was used for the remainder of this study unless otherwise noted (Fig. 1d).

The chemical structure of osas#9 is unusual in that it includes the neurotransmitter octopamine as a building block (Fig. 1a). Because both octopamine, and the biosynthetically related tyramine, play important roles in orchestrating starvation responses, we investigated receptors of octopamine (ser-3, ser-6, and octr-1) and tyramine (tyra-2, tyra-3, ser-2, and ser-3) for involvement in the osas#9 response (Fig. 2a). We found that avoidance to osas#9 is largely abolished in a tyra-2 loss of function (lof) mutant, whereas osas#9 avoidance was largely unaffected in the other neurotransmitter receptor mutants (Fig. 2a). We confirmed this phenotype was a result of the loss of tyra-2 by testing a second tyra-2 lof allele (Fig. 2b), and by neuron-targeted knockdown of tyra-2 (Supplementary Fig. 2A, B).

TYRA-2 is a G protein-coupled receptor (GPCR) that has been shown to bind tyramine with high affinity and, to a lesser extent, octopamine. To exclude the possibility that tyra-2 is necessary for general avoidance behaviors, we subjected tyra-2 lof animals to three well-studied chemical deterrents: sodium dodecyl sulfate (SDS), copper chloride (CuCl2), and glycerol. No defects were found in tyra-2 lof animals’ ability to respond aversively to these deterrents (Fig. 2c). This indicates that tyra-2 is specifically required for osas#9 avoidance, and is not part of a generalized unsensory avoidance response circuit. To determine the presence of receptors other than TYRA-2 that contribute to the sensation of osas#9, we exposed wild type and tyra-2 lof animals to increasing concentrations of osas#9 (Fig. 2d). Wild-type worms avoided osas#9 at all concentrations tested, whereas neither tyra-2 lof mutant avoided osas#9 at 1 µM or 10 µM concentrations (Fig. 2d). However, at 100 µM, both tyra-2 lof strains exhibited robust avoidance to osas#9, suggesting that other receptors respond to osas#9 at non-physiological concentrations (Fig. 2d).

Since the response to osas#9 is dependent on physiological state, we examined whether tyra-2 transcript levels changed under starvation conditions using quantitative RT-qPCR. Starved worms exhibited a nearly two-fold increase in tyra-2 expression (Fig. 2e).

Next we asked whether tyramine signaling is required for the osas#9 avoidance response, as tyra-2 is known to bind to endogenous tyramine. We assayed two tdc-1 lof mutants, which lack the ability to synthesize tyramine. We observed that the behavioral response to osas#9 was unaffected in animals lacking tyramine biosynthesis (Fig. 2f), demonstrating that the role of TYRA-2 in osas#9 avoidance is independent of tyramine, suggesting that TYRA-2 may be involved in perception of a ligand other than tyramine in promoting the aversive response to osas#9.
tyra-2 is required in the ASH neurons for osas#9 sensation. To better understand its role in the osas#9 aversion pathway we then asked where tyra-2 is expressed and localized. For this purpose, we designed a tyra-2 translational fusion construct consisting of the entire genomic locus, including 2 kb upstream, fused to GFP (ptyra-2::tyra-2::GFP). We observed TYRA-2 expression in four sensory neurons: ASH, ASE, ASG, and ASI (Fig. 3a); as well as the pharyngeal motor neuron, NSM. These results are in agreement with previous tyra-2 expression studies24 (Fig. 3a). We laser-ablated individual amphid sensory neurons to determine if a ablated tyra-2 expressing sensory neuron is required for the response. This revealed that ASH neurons are required for osas#9 response, whereas ablation of other neurons did not have a strong effect (Fig. 3b). We observed a slight reduction in the magnitude of the osas#9 aversive response in ASE and ASI laser-ablated animals (Fig. 3b), although ASH/ASE and ASH/ASI double ablated animals did not differ in response from animals with ASH ablated alone, and ASE/ASI ablated animals did not differ from ASE or ASI alone (Fig. 3b). To determine whether other sensory neurons played a role in mediating osas#9 avoidance, we tested genetic ablation lines of ASH, ASE, and ASI neurons31–34. We observed that at all tested concentrations, only the ASH genetic ablation line showed complete abolishment of osas#9 avoidance (Supplementary Fig. 3a–c). With the laser ablation studies, we observed a slight decrease in osas#9 avoidance in ASE and ASI ablated animals (Supplementary Fig. 3a–c). Ablation of neurons not expressing tyra-2 did not result in any defects in the response to osas#9 (Supplementary Fig. 3d). Our data implies that osas#9 is primarily sensed by the ASH sensory neurons, while the ASE and ASI sensory neurons may contribute to the sensation, possibly by sensitizing ASH sensory neurons or by regulating downstream interneurons within the osas#9 response circuitry35.

To further elucidate the role of the ASH sensory neurons and TYRA-2 in osas#9 sensation, we utilized a microfluidic olfactory imaging chip that enables detection of calcium transients in sensory neurons36,37. We observed that upon exposure to 1 µM osas#9, wild type animals expressing GCaMP3 in the ASH neurons exhibit robust increase in fluorescence (Fig. 3c, d and Supplementary Movie 1). Worms lacking tyra-2 displayed no fluorescence change upon osas#9 exposure (Fig. 3c, d). Exposure of worms to different concentrations of osas#9 elicited calcium changes in the ASH sensory neuron, correlating with avoidance responses observed at those concentrations (Fig. 3e, f, Supplementary Fig. 1b).

To test whether the observed ASH calcium signals are the result of direct sensation of osas#9 or are induced indirectly in response to osas#9 sensation in other neurons, such as ASE or ASI (Fig. 3b), we used genetic mutants that disrupted either synaptic signaling (unc-13)38 or peptidergic signaling (unc-31)39 (Supplementary Fig. 3e, f). In both unc-13 and unc-31 mutants, ASH neurons still displayed an increase in calcium levels upon osas#9 stimulation, indicating that ASH neurons can respond to osas#9.
osas#9 independent of feedback signals. However, the magnitude of osas#9-evoked ASH responses was significantly reduced in both unc-13 and unc-31 mutants. These data indicate that synaptic and peptidergic mechanisms contribute to the magnitude of the ASH response, suggesting that a distributed circuit modulates osas#9 dynamics, potentially involving other neurons such as ASE and ASI, as observed for other chemical stimuli in C. elegans.

To test whether ASH neurons can detect other small molecules, we used an unrelated ascaroside, ascr#3, which elicits male attraction in C. elegans. Stimulating ASH neurons with ascr#3 did not result in any calcium transients (Supplementary Fig. 3G, c–f).

Avoidance response to osas#9 and related stimuli in response to related stimuli:

- Wild type vs. solvent control
- Wild type vs. osas#9
- Mutants vs. solvent control
- Mutants vs. osas#9

- SDS 1%
- CuCl₂ 1 mM
- Glycerol 1M
- Wild type vs. solvent control
- Wild type vs. osas#9
- Mutants vs. solvent control
- Mutants vs. osas#9

Fed vs. Starved conditions:

- Relative tyra-2 expression
- Avoidance response

- Wild type vs. solvent control
- Wild type vs. osas#9
- Mutants vs. solvent control
- Mutants vs. osas#9

To further explore the role of differentially expressed genes, we examined the expression of genes related to Avoidance Index (e.g., tdc-1, n3419, n3420) in starved and fed conditions.
Given that tyramine and octopamine are known ligands of TYRA-2, we also tested whether these neurotransmitters elicit aversive responses in *C. elegans*. Both biogenic amines elicit aversive behaviors at non-physiological concentrations much higher than required for osas#9, i.e., 1 mM for tyramine and octopamine compared to 1 µM for osas#9 (Fig. 3g, Supplementary Fig. 4A). Similarly, this high concentration of tyramine was required to elicit calcium transients in ASH::GCaMP3 line, whereas lower concentrations (1 µM) did not (Fig. 3h, i). Worms exposed to 1 mM octopamine also only displayed minimal changes in calcium transients (Supplementary Fig. 4B, C).

Previous studies have shown that both tyramine and octopamine inhibit serotonin-mediated food-depended aversive responses to dilute octanol via specific GPCRs. To test whether tyramine inhibits osas#9 sensation, we performed a competition experiment where we exposed worms to different ratios of tyramine and osas#9 (Supplementary Fig. 4D). Wild type worms exposed to equimolar concentrations of tyramine and osas#9 displayed robust avoidance mediated by the TYRA-2 receptor (Supplementary Fig. 4D). Mixtures containing very high (1 mM) concentrations of tyramine elicited aversion in both wild type and *tyra-2* lof worms, as in the experiments with pure tyramine (Supplementary Fig. 4D). These results indicate that expression of the TYRA-2 receptor in the ASH sensory neurons is specifically involved in the response to osas#9.

**tyra-2 expression confers the ability to sense osas#9.** Since expression of *tyra-2* in the ASH sensory neurons is required for osas#9 elicited calcium transients, we asked whether *tyra-2* expression in the ASH neurons is sufficient to rescue the osas#9 behavioral response in *tyra-2* lof animals. Expression of *tyra-2* under the *nhr-79* promoter, which resulted in expression in the ASH and ADL neurons, fully restored osas#9 avoidance (Fig. 4a, b). We then tested whether rescue of *tyra-2* expression rescues the neurophysiological properties in ASH neurons upon osas#9 exposure. We generated a line expressing GCaMP3 in the ASH neuron in the transgenic rescue line and observed that osas#9 exposure elicited calcium transients similar to wild type animals (Fig. 4c, d).

To demonstrate sub-cellular localization of the TYRA-2 protein in the sensory neurons, we injected the translational reporter generated in Fig. 3a at a lower concentration (1 ng/µl) into *tyra-2* lof animals, as this has been found to improve reporter localization in the sensory cilia (Maurya and Sengupta, personal communication). The worms expressing the transgene displayed sub-cellular localization in the ASH sensory cilia (Fig. 4e). osas#9 aversion is rescued in these animals, indicating that the transgene is functional (Fig. 4f). These results affirm that the aversive behavioral response to osas#9 is dependent on TYRA-2 localization in the ASH neuronal cilia.

Previous studies in *C. elegans* indicate that behavioral responses (such as aversion or attraction) elicited by an odorant are specified by the olfactory neuron in which the receptor is present, rather than by the olfactory receptor itself. We asked whether driving TYRA-2 receptor expression in other sensory neurons will drive behavioral response to osas#9. For this purpose, we ablated the ASH neurons in the *p{nhr-79::tyra-2}* strain, in which *tyra-2* is expressed in the ASH and ADL neurons (Fig. 4a, b). ADL neurons have also been shown to detect aversive stimuli. We found that these ASH-ablated transgenic animals still avoid osas#9, similar to ADL ablated worms from this rescue line (Fig. 5a, b). Ablation of both the ASH and ADL neurons in this strain abolished the avoidance response (Fig. 5a, b). This implies that misexpression of *tyra-2* in the ADL neurons is sufficient to confer avoidance to osas#9.

We then asked whether expression of *TYRA-2* in AWA neurons, which are generally involved in attractive responses to chemical cues, would switch the behavioral valence of osas#9, resulting in attraction to osas#9, instead of aversion. Misexpression of *tyra-2* in the AWA sensory neurons resulted in reprogramming of the behavioral circuits, promoting attraction to the normally aversive compound osas#9.

**Screen for receptors required to mediate osas#9 avoidance.** To identify putative receptors required to mediate osas#9 chemotaxis, we performed a forward genetic screen by generating a transgenic rescue line and observed that osas#9 exposure elicited calcium transients, we asked whether rescue of expression in the ASH neurons is sufficient (Fig. 4f). These results affirm that the aversive behavioral response to osas#9 is dependent on TYRA-2 receptor expression in other sensory neurons as required for osas#9 avoidance. 

**Tyra-2 lof animals are defective in osas#9 avoidance response.** The expression of *tyra-2* receptor is dependent on the physiological state of the animal. RT-qPCR analysis starved animals indicates a nearly twofold upregulation of *tyra-2*. Data are displayed as the ratio of endogenous *tyra-2* mRNA to *amo-1* mRNA from three independent RT-qPCR experiments. n = 3 trials. osas#9 avoidance response is not dependent on endogenous tyramine. Two different alleles of *tdc-1* lof animals (n3419 and n3420) which are deficient in tyramine biosynthesis, exhibit normal response to osas#9. n ≥ 7 trials. Data presented as mean ± S.E.M.; *p < 0.05, **p < 0.01, ***p < 0.001; one factor ANOVA with Sidak’s multiple comparison posttest except for 2D and 2E, where Student’s t-test was used.
gpa-6 is necessary in ASH for osas#9 avoidance. Since expression of the GPCR, TYRA-2, in ASH neurons is required for the osas#9 response, we sought to identify the Gα subunit necessary for osas#9 avoidance. Eight of the twenty-one Gα proteins are expressed in subsets of neurons that include the ASH sensory pair (gpa-1, gpa-3, gpa-6, gpa-11, gpa-13, gpa-14, gpa-15, and odr-3)\textsuperscript{46,47}. We tested mutants for each of those eight Gα subunits for their response to osas#9 (Fig. 6a), and found that gpa-6 lof animals do not avoid osas#9 (Fig. 6a). To determine whether gpa-6 is necessary in ASH sensory neurons to mediate osas#9 responses, we expressed gpa-6 using pnhr-79 in the ASH neurons in a gpa-6 lof background. These animals displayed osas#9
Fig. 3 TYRA-2 is required in ASH for sensation of osas#9. a Cellular localization of TYRA-2 in sensory neurons. Expression is seen in ASE, ASG, ASH, and NSM neurons (×40 magnification, scale bar denotes 10 μm). b Chemosensory neurons required for osas#9 response. Neurons expressing tyra-2 reporter were ablated using laser microbeam. ASH ablations resulted in abolished response to osas#9 that was indistinguishable from solvent control. ASE and ASI ablated animals showed reduced but not complete loss of avoidance. n ≥ 3 trials (≥10 ablated animals precondition). c, d Calcium dynamics of ASH upon osas#9 exposure. e ASH::GCaMP3 animals (black) exhibit calcium transients when exposed to 1 μM osas#9. tyra-2 lof animals (red) did not display a change in fluorescence upon stimulation. Shaded region gray depicts time animals were subjected to osas#9, n = 10 animals. d Maximum fluorescence intensity before (solvent control, gray) and during exposure to 1 μM osas#9 of ASH::GCaMP3 animals (black) and tyra-2 lof animals (red). e osas#9 elicits calcium transients in ASH neurons at a broad range of concentrations: 1 μM (black), 1 nM (red), 100 nM (blue), 1 μM (orange), 10 μM (magenta), 100 μM (cyan), n ≥ 10 animals per condition. f Maximum fluorescence intensity before and during exposure to varying osas#9 concentrations. g Tyramine elicits avoidance only at high concentrations in wild-type animals, n ≥ 5 trials. h Calcium dynamics in ASH upon exposure to different concentrations of tyramine, for ASH::GCaMP3 1 μM (magenta) and 1 nM (cyan) and tyra-2 lof 1 mM tyramine (red). Tyramine exposure resulted in a significant increase in calcium transients in ASH at concentrations of 1 μM, but not 1 nM, n ≥ 10 animals. i Maximum fluorescence intensity before (solvent control) and during exposure to varying tyramine concentrations for ASH::GCaMP3 animals and tyra-2 lof animals. Data presented as mean ± S.E.M.; *p < 0.05, **p < 0.01, ***p < 0.001. Figure 3b, one factor ANOVA with Sidak’s multiple comparison posttest. Figure 3d, f, i Student’s t-test was used to compare the solvent control to stimulus max peak fluorescence.

Discussion

How does a worm survive in changing environmental and physiological conditions? Given C. elegans’ complex ecology and boom-and-bust lifestyle, worms need to make frequent adaptive developmental and physiological choices48. The octopamine-derived pheromone, osas#9, secreted in large quantities by L1 larvae under starvation conditions, appears to promote escape away from unfavorable conditions (Fig. 7). Here we show that this pheromone is detected by the GPCR, TYRA-2, a canonical neurotransmitter receptor expressed in the ASH sensory neurons. To our knowledge, this is the first instance in which a “repurposed internal receptor” partakes in pheromone perception. Notably, octopamine, the distinguishing structural feature of osas#9, has been implicated in responses to food scarcity in invertebrates, including insects49–51, C. elegans22,52–55, and molluscs56. Our findings indicate that worms navigate adverse environmental conditions, in part, using social communication networks that employ signaling molecules and receptors derived from relevant endocrine signaling pathways (Fig. 7).

TyRA-2 has previously been shown to contain the conserved Asp332 residue required for amine binding, allowing the receptor to bind tyramine with high affinity and, to a lesser extent, octopamine24. In contrast, osas#9 lacks a basic amine, instead containing an amide, as well as an acidic sidechain. These chemical considerations suggest that TYRA-2 may facilitate osas#9 perception by interacting with another GPCR that directly binds to osas#9. Several studies have already demonstrated thatGPCRs involved in ascaroside perception act as heterodimers18, and it is possible that another receptor expressed in the ASH, ADL, and AWA neurons directly interacts with TYRA-2 and is responsible for binding osas#9. Furthermore, the neurotransmitter tyramine has been shown to activate the Gα/o protein-coupled tyramine receptor, TYRA-2, in different sensory and interneurons to mediate different behaviors such as multisensory decision making and feeding suppression57,58. Tyramine released from the RIM interneurons activates the TYRA-2 receptor in ASH neurons to mediate threat tolerance in a positive feedback loop58. Similarly, TYRA-2 functions in AIM interneurons to respond to tyramine release from the RIM interneurons in the mediation of feeding suppression57. Our results show that endogenous tyramine signaling is not directly involved in the response to osas#9, but instead other neurons and neuromodulatory signaling pathways participate in shaping the osas#9 response. Such modulation of the osas#9 response circuitry remains to be investigated.

Exactly how key innovations in metazoan signaling complexity evolved from pre-existing machineries remains to be elucidated59. Neurotransmitter signaling is typically inter-cellular, i.e., facilitating cell-to-cell communication, involves highly regulated biosynthesis of specific small molecules (e.g. biogenic amines), their translocation (either by way of diffusion or through active transport), and perception by dedicated receptors60. This mode of signaling is strikingly similar to pheromone-mediated communication systems, which rely on highly specific production and perception of small molecule ligands for inter-organismal signaling61. During evolution, it stands to reason that some machinery from inter-cellular signaling would also be utilized for inter-organismal signaling. Co-option of such signaling systems has been observed in both invertebrates (C. elegans), where a nicotinic acetylcholine receptor senses choline62, and vertebrates (such as mice and zebrafish), where some metabolotropic neurotransmitter receptors act as sensors to detect amino acids in the environment63–65.

In summary, our findings demonstrate that the tyramine receptor, TYRA-2, functions in the chemosensation of osas#9, a neurotransmitter-derived inter-organismal signal. These results reveal the participation of both neurotransmitter biosynthesis and reception in inter-cellular as well as inter-organismal signaling. Hence, it appears that evolution of an inter-organismal communication pathway co-opted both a small molecule (octopamine), and a related receptor (TYRA-2) for mediating starvation-dependent dispersal in C. elegans (Fig. 7), suggesting that such co-option may represent one mechanism for the emergence of new inter-organismal communication pathways.

Methods

Avoidance drop test. The tail end of a forward moving animal was subjected to a small drop (~5 nl) of solution, delivered through a hand-pulled 10 μl glass capillary tube. The solution, upon contact, was drawn up to the amphid sensory neurons via capillary action. In response, the animal either continued its forward motion (scored as “no avoidance response”), or displayed an avoidance response within 4 s66. The avoidance response was characterized a reversal if the behavior consisted of at least one half of a complete “head swing” followed by a change in direction of at least 90° from the original vector. For quantitative analysis, an avoidance response was scored as “1” and no response as “0”. The avoidance index was calculated by dividing the number of avoidance responses by the total number of trials. Each trial was done concurrently with osas#9 and a solvent control.
Control animals and strains containing transgenes in various genetic backgrounds were prepared using common M9 buffer to wash and transfer a plate of animals to a microcentrifuge tube where the organisms are allowed to settle. The supernatant was removed and the animals were resuspended and allowed to settle again. The supernatant was again removed and the animals transferred to an unseeded plate. After 1 h, young adult animals were subjected to the solvent control and chemical of interest at random, with no animal receiving more than one drop of the same solution. Refed animals were transferred to a seeded plate with M9 buffer, and after the allotted time, transferred to an unseeded plate and tested after 10 minutes.

Ablated and extrachromosomal transgenic animals and controls were gently passed onto an unseeded plate and allowed to crawl around. They were then again
gently passed to another unseeded plate to minimalize bacterial transfer. Ablated animals were tested three times with the solvent control and solution of interest with 2 min intervals between drops.

**Strains and plasmids.** tyra-2 rescue and misexpression plasmids were generated using MultiSite Gateway Pro Technology and injected into strain FX01846 tyra-2 (tm1846) with co-injection marker pelt-2mCherry by Knudra Transgenics. The promoter atB inserts were generated using PCR and genomic DNA or a plasmid. The tyra-2 insert was isolated from genomic DNA using attB5 ggttatcgtgtggagaa and attB2tgcccctttctttcttt. PDONR221 p1-p5r and PDONR221 P5-P2 donor vectors were used with atB inserts. The resultant entry clones were used with the destination vector pLR305 and pLR306.
Fig. 5 Ectopic expression of tyra-2 confers the ability to respond to osas#9. a Misexpression of tyra-2 in ADL neurons confers avoidance behavior in response to osas#9. nhr-79 promoter driving tyra-2 expression in ASH and ADL sensory neurons rescues osas#9 avoidance. Ablation of either ADL or ASH neurons does not affect osas#9 avoidance in the rescue lines, suggesting that misexpression of tyra-2 in ADL neurons is sufficient for osas#9 response. Ablation of both ASH and ADL completely abolished avoidance. n ≥ 5 trials. b Schematic illustration of cellular ablations in the transgenic rescue lines expressing tyra-2 under the nhr-79 promoter. c Animals with reprogrammed AWA sensory neurons in tyra-2 lof background do not avoid 1 µM osas#9. n ≥ 4 trials. d AWA neurons (black) do not exhibit calcium transients in response to 1 µM osas#9, while reprogrammed AWA::tyra-2 neurons (red) are hyperpolarized upon exposure (gray-shaded region), n = 10 animals. e Maximum fluorescence intensity in transgenic worms before (solvent control) and during exposure to 1 µM osas#9. f Schematic illustration of the leaving assay to measure osas#9 attraction. g Wild type (black), tyra-2 lof (red), and AWA::tyra-2 lines (1 [magenta], 2 [cyan]) were subjected to 10 pM osas#9 in the leaving assay. Wild-type animals left the osas#9 solution spot quicker than the tyra-2 lof animals, whereas the misexpression lines remained closer to osas#9, n ≥ 3 trials. h Reprogrammed AWA::tyra-2 animals have an increased reversal rate in comparison to both wild type and tyra-2 lof animals in 10 pM osas#9, n ≥ 3. Data presented as mean ± S.E.M; * p < 0.05, ** p < 0.01, *** p < 0.001. One factor ANOVA with Sidak's multiple comparison posttest.

Fig. 6 GPA-6 functions in ASH sensory neurons to mediate osas#9 response. a Screen of mutations in Gα subunits resulted in identification of the Gα subunit, gpa-6, which was defective in avoidance response to osas#9, n ≥ 3 trials. b Expression of gpa-6 in ASH neurons using nhr-79 promoter reconstituted avoidance response similar to wild-type animals, n ≥ 3 trials. c gpa-6 localizes to the soma and cilia in ASH neurons. Translational fusion of the entire gpa-6 genomic region displayed localization of the subunit to the soma of AWA, AWB, and ASH neurons. In addition, we also observed ciliary localization in ASH neurons (×40 magnification, scale bar denotes 10 µm). Data presented as mean ± S.E.M; * p < 0.05, ** p < 0.01, *** p < 0.001. One factor ANOVA with Sidak’s multiple comparison posttest.

Fig. 7 osas#9 serves as a dispersal cue for C. elegans. An animal navigating its environment encounters a food source, where offspring grow and reproduce rapidly, eventually depleting their food source. Eggs hatch on the depleted food patch and halt development as L1 arrest animals. L1 arrest animals secrete the aversive compound, osas#9, signaling for conspecifics to disperse away from the unfavorable condition.
For AWA expression, a 1.2-kb odr-10 promoter was isolated from genomic DNA using primers attB1 (5′-ttttctgatgaagccttttgctgtagaagccttttgctgtag) and attB5 (5′-attttctgatgaagccttttgctgtagaagccttttgctgtag). Entry clones were used with destination vector pLR306 resulting in pLR306:tyrR2::RFP and co-injected with pelt-2::mCherry into FX01846.

For ASH expression, a 3-kb nhr-79 promoter was isolated from genomic DNA using primers attB1 (5′-ttttctgatgaagccttttgctgtagaagccttttgctgtag) and attB5 (5′-attttctgatgaagccttttgctgtagaagccttttgctgtag). Entry clones were used with destination vector pLR306 resulting in pLR306:tyrR2::RFP and co-injected with pelt-2::mCherry into FX01846.

For ASH expression, a 3-kb nhr-79 promoter was isolated from genomic DNA using primers attB1 (5′-ttttctgatgaagccttttgctgtagaagccttttgctgtag) and attB5 (5′-attttctgatgaagccttttgctgtagaagccttttgctgtag). Entry clones were used with destination vector pLR306 resulting in pLR306:tyrR2::RFP and co-injected with pelt-2::mCherry into FX01846.

Chemical compounds. ascR3# and osaR9# were synthesized as previously described.

RNA interference. RNAi knockdown experiments were performed by following the RNAi feeding protocol found at Source Bioscience (https://www.sourcebiosciences.com/products/life-sciences-research/clones/rnai-resources/c-elegans-rnai-collection-ahringer/). The RNAi clones (F01E11.5, F14D12.6, and F22A11.1) originated from the Vidal Library, were generously provided by the Ambros Lab at UMass Medical School. We observed that RNAi worked best when animals were cultured at 15 °C. We used VHA24 (rde-1;lin-15B(hd126)) for the RNAi studies, as it has been previously shown to be sensitive to neuronal RNAi. 

Laser ablations. Laser ablations were carried out using DIC optics and the MicroPoint laser system. L1 worms were immobilized on 2% agarose on a glass slide using 1 mM sodium azide. The neurons of the L1 animals were identified and ablated at the nucleus by pulses of laser. Animals were removed from the slide and allowed to recover. Ablated animals were assayed 72 hours later, at the young adult stage. All ablated animals were tested in parallel with control animals that were treated similarly as ablated animals but were not exposed to the laser microbeam.

Imaging. Translational fusion animals were prepared for imaging by mounting them to a 4% agar pad with 10 mM levamisole on a microscope slide. Animals were imaged using a Nikon Multispectral Multimode Spinning Disk Confocal Microscope, courtesy of the Department of Neurobiology at University of Massachusetts Medical School, Worcester. Calcium imaging was performed by using a modified olfactory chip described in Reilly et al. For imaging, worms were treated in a similar way as the behavioral assays. Worms were starved for an hour when imaging with osaR9. A young adult animal was immobilized in a PDMS olfactory chip with its nose subject to a flowing solution. Animals were imaged at x40 magnification for 30 s, and experienced a 10 s pulse of osaR9, tyramine, octopamine, or ascR3# in between the solvent control. Each animal was exposed to the stimulus up to three times; multiple exposures to the chemical did not show a significant difference in response between exposures. Some fluorescence from GCaMP3 was measured using ImageJ. Background subtraction was performed for each frame to obtain the value ΔF. Change in fluorescence (ΔF/ΔF0) was calculated by dividing the ΔF value of each frame by F0. F0 was calculated as the average ΔF of 10 frames prior to stimulus exposure. ΔF/ΔF0 was calculated from the ratio of subtracting 1 pixel (approximately 100%) these calculations were then plotted over the duration of the experiment.

Quantitative RT-qPCR. RNA was isolated from individual animals, either freshly removed from food or after four hours of starvation using Proteinase K buffer. cDNA was subsequently synthesized using the Maxima H Minus First Strand cDNA Synthesis Kit. RTaq Universal SYBR Green Supermix was used for amplification with the Applied Biosysten 7500 Real Time PCR. Transcript levels of the positive control gene, actin, were determined to be 97.4% for tyrR2-primes (GAGAGAGAAGAGATGCCTGGTATCAATCCTGCAGTTTTCAAGCGAAACAAAAAATTTTTCAAA) and 101.8% for the reference gene actin-1 (GGAGAGAATGCGACGATGTGTCATGCATCTCCAGG).

Locomotion. Speed: Five animals were gently transferred to a 35-mm plate and filmed for 20 minutes. Movies were generated using the Wormtracker system by MBF Bioscience. Movies were then analyzed and average speed was computed using software (WormLab). The authors declared that all data supporting the findings of this study are available within the paper and its supplementary information files. The source data underlying all figures are provided as a Source Data file.

Received: 27 September 2018 Accepted: 28 June 2019 Published online: 18 July 2019

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