

Neuroendocrine Gene Expression Coupling of Interoceptive Bacterial Food Cues to Foraging Behavior of *C. elegans*

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Abstract

Summary

Animal internal state is modulated by nutrient intake, resulting in behavioral responses to changing food conditions. The neural mechanisms by which internal states are generated and maintained are not well understood. Here, we show that in the nematode *Caenorhabditis elegans*, distinct cues from bacterial food – interoceptive signals from the ingestion of bacteria and gustatory molecules sensed from nearby bacteria – act antagonistically on the expression of the neuroendocrine TGF-beta ligand DAF-7 from the ASJ pair of sensory neurons to modulate foraging behavior. A positive-feedback loop dependent on the expression of *daf-7* from the ASJ neurons acts to promote transitions between roaming and dwelling foraging states and influence the persistence of roaming states. SCD-2, the *C. elegans* ortholog of mammalian Anaplastic Lymphoma Kinase (ALK), which has been implicated in the central control of metabolism of mammals, functions in the AIA interneurons to regulate foraging behavior and cell-non-autonomously control the expression of DAF-7 from the ASJ neurons. Our data establish how a dynamic neuroendocrine *daf-7* expression feedback loop regulated by SCD-2 functions to couple sensing and ingestion of bacterial food to foraging behavior. We further suggest that this neuroendocrine feedback loop underlies previously characterized exploratory behaviors in *C. elegans*. Our data suggest that the expression of *daf-7* from the ASJ neurons contributes to and is correlated with an internal state of “unmet need” that regulates exploratory foraging behavior in response to bacterial cues in diverse physiological contexts.

eLife assessment

This **important** manuscript focuses on the mechanisms by which food signals and food ingestion modulate animal foraging. The authors provide **convincing** support for the interesting idea that chemosensory and interoceptive signals converge on transcriptional regulation of the TGF-beta ligand DAF-7 in a single pair of *C. elegans* chemosensory neurons (ASJ) to regulate behavior. Their studies implicate a conserved signaling molecule, ALK, in this regulation, suggesting a conserved link between food cues and the neuroendocrine control of foraging behavior.

Introduction

Internal states, such as fear, arousal, and hunger, are shaped by the integration of information about internal and external conditions and can result in the modulation of various physiological and behavioral outputs (Flavell et al., 2022 [↗](#)). As animals encounter different food environments, information about internal nutritional status and external food quality can elicit transitions between internal states that either favor the exploration of new places or exploitation of the current environment. Many animals, including mammals, zebrafish, *Drosophila melanogaster*, and the roundworm *Caenorhabditis elegans*, increase exploration in response to food deprivation in order to increase chances of a food encounter (Ben Arous et al., 2009 [↗](#); Connolly, 1966 [↗](#); Gutman et al., 2007 [↗](#); Herbers, 1981 [↗](#); Johnson et al., 2020 [↗](#); Overton & Williams, 2004 [↗](#); Russell et al., 1987 [↗](#)). However, the mechanisms behind how animals couple changes in food availability to internal states that influence foraging behavior are not well understood. Insight into the cellular and organismal mechanisms governing internal states may enhance understanding of the dysregulation of internal states that is thought to contribute to many human psychiatric and neurological diseases (Flavell et al., 2022 [↗](#); Yap & Greenberg, 2018 [↗](#)).

C. elegans forage for microbes that grow on decaying organic matter, where they encounter fluctuations in not only the quantity of nutritious bacterial food available but also in the quality and pathogenicity of this bacterial food (Kim & Flavell, 2020 [↗](#)). As they navigate their food environments, *C. elegans* exhibit two-state foraging and feeding behavior known as roaming and dwelling (Ben Arous et al., 2009 [↗](#); Flavell et al., 2013 [↗](#); Flavell et al., 2020 [↗](#); Fujiwara et al., 2002 [↗](#)). On abundant nutritious food, animals spend about 80% of their time dwelling and 20% of their time roaming; as food becomes scarcer or lower in quality, animals increase the proportion of their time roaming (Ben Arous et al., 2009 [↗](#)). The internal states that underlie *C. elegans* roaming and dwelling responses to changing food conditions present an experimentally tractable paradigm in which to study how internal states are regulated. Furthermore, the shared modulators of internal states and high degree of genetic conservation between *C. elegans* and humans suggest that understanding *C. elegans* feeding and foraging behavior could have relevant implications for human health and disease.

Aided by a complete connectome (White et al., 1986 [↗](#)), a growing body of work has examined neural circuits and their effects on foraging behavior in *C. elegans* (Ji et al., 2021 [↗](#); Pradhan et al., 2019 [↗](#)). The excitation or inhibition of neurons in these circuits elicits rapid behavioral modulation as well as persistent behavioral states through recurrent neuronal firing. Neuronal dynamics are additionally influenced by neuromodulators (Bargmann, 2012 [↗](#)). Neuromodulators play a key role in mediating internal states by functioning at titratable levels over longer timescales and farther distances than direct synaptic signaling (Flavell et al., 2022 [↗](#); Sengupta, 2013 [↗](#)). For instance, the activation of a small group of neurons can induce transitions between roaming and dwelling states through conserved serotonin and pigment dispersing factor signaling

(Flavell et al., 2013 [↗](#); Ji et al., 2021 [↗](#)). Other neuromodulators such as dopamine and octopamine can further influence foraging behavior and food-dependent locomotion (Churgin et al., 2017 [↗](#); Oranth et al., 2018 [↗](#); Sawin et al., 2000 [↗](#)). Activity-dependent gene expression in neurons has been established to have key roles in the development and plasticity of neuronal circuits, but less is known about how changes in neuronal gene expression may shape internal states driving behavior (Yap & Greenberg, 2018 [↗](#)). As transcription occurs on a slower timescale than neuronal firing or neuromodulator release, dynamic gene expression could be important in regulating the persistence of internal states.

We have been studying the dynamic temporal and neuron-specific expression of *daf-7*, which encodes a TGF-beta ligand that is involved in the neuroendocrine regulation of a diverse range of behaviors in *C. elegans*, including the dauer developmental decision, longevity, metabolism, and feeding and foraging behavior (Ben Arous et al., 2009 [↗](#); Greer et al., 2008 [↗](#); Ren et al., 1996 [↗](#); Schackwitz et al., 1996 [↗](#); Shaw et al., 2007 [↗](#)). Expression of *daf-7* is restricted to a limited set of sensory neurons, including the ASI neurons (Meisel et al., 2014 [↗](#); Ren et al., 1996 [↗](#); Schackwitz et al., 1996 [↗](#)). DAF-7 expression from the ASI neurons was shown to respond to changing environmental conditions, such as crowding and food levels (Entchev et al., 2015 [↗](#); Ren et al., 1996 [↗](#); Schackwitz et al., 1996 [↗](#)). Previously, we observed that highly dynamic *daf-7* expression can be observed in the ASJ neurons, with induction of expression in response to secondary metabolites produced by pathogenic *Pseudomonas aeruginosa* PA14, which is necessary for pathogen avoidance behavior (Meisel et al., 2014 [↗](#)). In addition, we have shown that upon the onset of reproductive maturity, male *C. elegans* upregulate *daf-7* expression in their ASJ neurons to promote male mate-searching behavior (Hilbert & Kim, 2017 [↗](#)).

Here, we have identified that the ingestion of bacterial food regulates the expression of a single gene in two neurons to shape internal state dynamics driving foraging behavior in *C. elegans*. We observed that *daf-7* transcription levels in the ASJ neurons couples foraging behavior to changes in bacterial food ingestion, under the control of the highly conserved receptor tyrosine kinase SCD-2/ALK. The relationship between gene transcription and behavioral states across organisms is largely uncharacterized (Yap & Greenberg, 2018 [↗](#)), and our results establish SCD-2/ALK-regulated dynamic *daf-7* expression as a gene-expression correlate and driver of internal states underlying foraging behavior in response to changing nutritional conditions.

Results

Ingested food inhibits *daf-7* expression in the ASJ neurons

Our lab has previously reported that when adult hermaphrodites are fed the normal *E. coli* OP50 food source, *pdaf-7::GFP* expression is restricted to the ASI chemosensory neurons (Hilbert & Kim, 2017 [↗](#); Meisel et al., 2014 [↗](#)). In contrast, we observed that when animals were fed OP50 treated with aztreonam, an antibiotic that causes the bacteria to form inedible long strands (Gruninger et al., 2008 [↗](#)), *pdaf-7::GFP* was expressed in both the ASI and ASJ neurons (Figures 1A [↗](#), 1C, 1D). These data suggested that ingestion of food inhibits *daf-7* expression in the ASJ neurons. However, we did not observe *daf-7* expression in the ASJ neurons in the complete absence of food (“Empty”), which indicated that some bacterial component of non-ingestible aztreonam-treated *E. coli* OP50 was required for the induction of *daf-7* in the ASJ neurons in the absence of ingested food (Figures 1A [↗](#), 1C, 1D). The non-ingestible food signal that results in the upregulation of *daf-7* expression in the ASJ neurons on aztreonam-treated food could be diffusible, volatile, or mechanosensory. To identify the nature of the non-ingestible food cue, we exposed animals to food that had been seeded on the lid of the plate (exposing animals to only the volatile food cues) (“Lid”) or underneath the agar (exposing animals only to diffusible food cues) (“Bottom”) in the absence of ingestible food and probed *daf-7* expression. We observed induction of *daf-7* in the ASJ neurons when OP50 is seeded under the agar of the plates, but not when present on the lid of the plate,

suggesting that a bacteria-derived food signal that diffuses through the agar is necessary for the induction of *daf-7* expression in ASJ in the absence of ingested food. (Figures 1A-F). Thus, these data suggest that in hermaphrodites feeding on *E. coli* OP50, there is an external water-soluble diffusible cue from the bacteria that stimulates *daf-7* expression from the ASJ neurons, but this expression is inhibited by a second bacteria-derived, interoceptive cue generated from the ingestion of bacteria (Figure 1B).

We next asked whether the inhibition of *daf-7* expression in the ASJ neurons by ingestion of bacterial food was mediated by the early detection of ingested food or the delayed nutritional effects of bacterial food consumption on organismal physiology. The kinetics of *pdaf-7::GFP* induction in the ASJ neurons when animals were moved from ingestible to non-ingestible food showed a rapid upregulation within 2-3 h for the GFP fluorescence in the ASJ neurons to become visible (Figure 1G). Given the delay for GFP folding and accumulation to visible levels, these data suggested that the absence of ingested food rapidly led to the induction of *daf-7* expression in the ASJ neurons. Recent work has described the interoceptive sensing of ingested food by the acid-sensing ion channels (ASICs) encoded by *del-3* and *del-7*, which are expressed in the minor neurites of the NSM neurons where they detect food in the pharynx and mediate behavioral slowing upon encountering food (Rhoades et al., 2019). We observed that *del-3;del-7* animals have elevated expression *daf-7* in the ASJ neurons on ingestible food but show wild-type levels of *daf-7* expression on non-ingestible food or no food (Figure 1H). These observations are consistent with the hypothesis that the detection of an interoceptive ingested food signal in the pharynx, mediated by DEL-3 and DEL-7, inhibits *daf-7* expression in the ASJ neurons.

***daf-7* expression in the ASJ neurons promotes roaming behavior**

Next, we sought to understand how changes in *daf-7* expression in the ASJ neurons in response to changing food conditions might modulate food-dependent behavior. Feeding *C. elegans* alternate between two distinct behavioral states known as roaming and dwelling (Ben Arous et al., 2009; Fujiwara et al., 2002). On nutritious food, wild-type animals spend most of their time dwelling, a feeding state marked by a low movement speed and high body bending angle. As food conditions worsen, animals will decrease the fraction of time dwelling and increase the fraction of time roaming, a foraging state marked by high speed and low curvature (Ben Arous et al., 2009). Prior analysis of roaming and dwelling has identified that animals increase the proportion of time roaming on aztreonam-treated food, and genetic analysis has revealed a role for DAF-7 in promoting roaming behavior, with *daf-7* animals spending a decreased fraction of time roaming compared to wild-type animals (Ben Arous et al., 2009). Using both worm-tracking software to quantify roaming and dwelling (WormLab, 2020) and an exploration assay to measure the general activity levels of animals (Flavell et al., 2013), see Materials and Methods, we observed that *daf-7(e1372)* and *daf-7(ok3125)* animals spent a markedly decreased proportion of time in the roaming state than wild-type animals, consistent with these prior findings (Figure 2A). To determine whether we could detect modulation of roaming and dwelling behaviors caused by changes in *daf-7* expression in the ASJ neurons, we adopted two complementary approaches. First, we found that in a *daf-7(ok3125)* background, rescue of *daf-7* cDNA under the ASJ-specific *trx-1* promoter partially restored the fraction of time animals spent in the roaming state (Figure 2B). Second, we examined exploratory behavior in animals that had *daf-7* deleted in the ASJ neurons, using a floxed allele of *daf-7* and Cre expressed under the *trx-1* promoter. We found that animals with an ASJ-specific *daf-7* deletion explored less of the lawn and spent less time roaming than animals with wild-type expression of *daf-7* (Figure 2C).

The relationships between ingested food and *daf-7* expression in the ASJ neurons, *daf-7* expression in the ASJ neurons and roaming, and roaming and amount of food ingested suggest a neuroendocrine gene expression positive-feedback loop that couples the ingestion of bacterial food to the modulation of foraging behavior through *daf-7* expression in the ASJ neurons (Figure 2D). When animals are on abundant edible food, the ingestion of this food inhibits *daf-7* expression in the ASJ neurons and attenuates the proportion of time in the roaming state. When

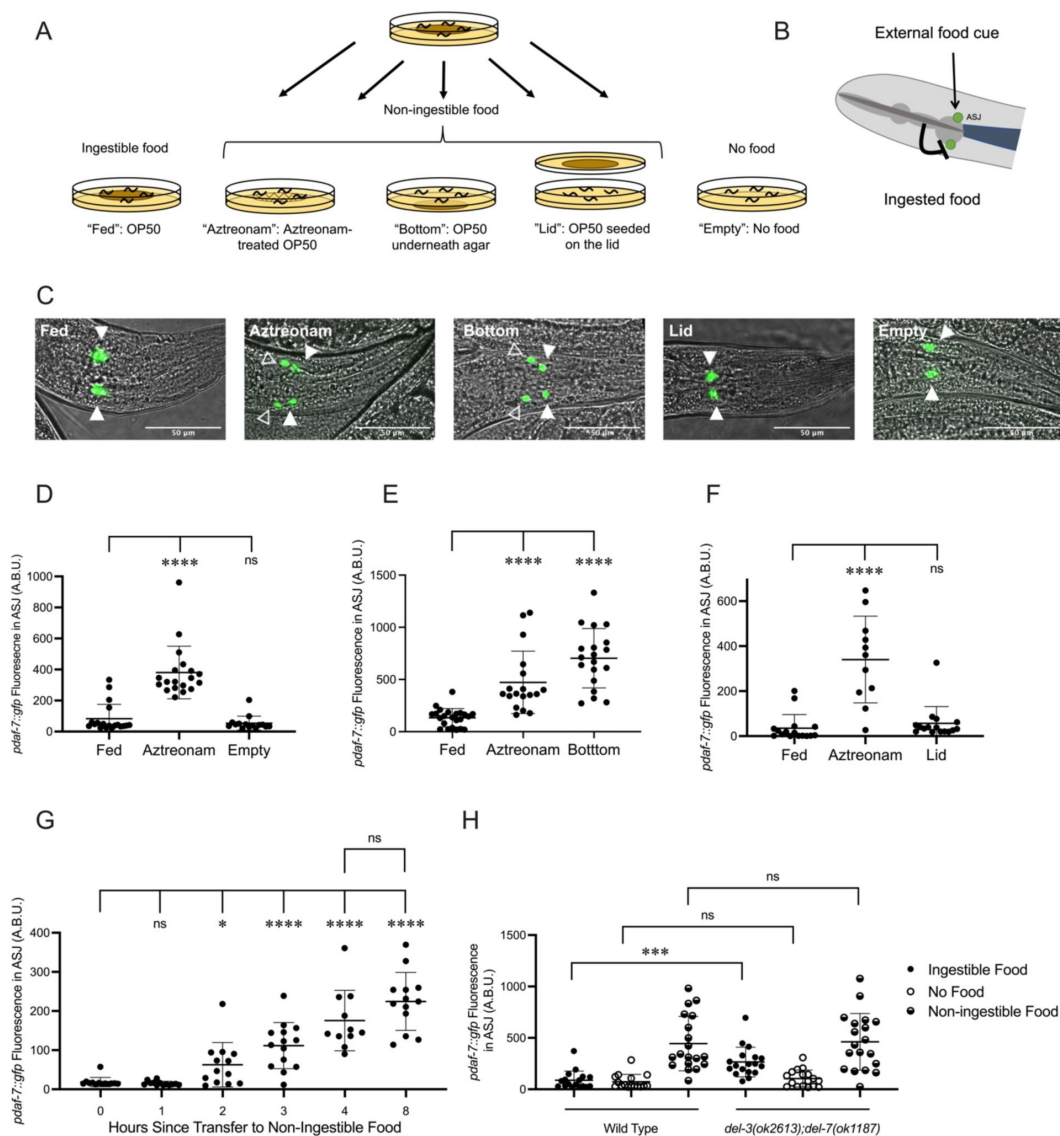


Figure 1.

Ingestion of bacterial food inhibits *daf-7* expression in the ASJ neurons.

A. Schematic of experimental exposure to varied food conditions. Animals were grown to adulthood on edible *E. coli* OP50, then transferred to various experimental food conditions: "Fed": *E. coli* OP50; "Aztreonam": OP50 treated with aztreonam; "Bottom": OP50 was seeded underneath the agar of the plate; "Lid": Animals placed on agar with no food but where food was seeded on a spot of agar on the inside of the lid of the plate; "Empty": no food.

B. Model for the convergence of external and ingested food signals on *daf-7* expression in the ASJ neurons.

C. *pdaf-7::gfp* expression pattern in animals under different food conditions, from left to right: "Fed," "Aztreonam," "Bottom," "Lid," "Empty." Filled triangles indicate the ASI neurons; open triangles indicate the ASJ neurons. Scale bar indicates 50 μ m.

D. E. F. Maximum fluorescence values of *pdaf-7::gfp* in the ASJ neurons of adult animals under various food conditions. Each point represents an individual animal, and error bars indicate standard deviation. **** $p < 0.0001$, ns not significant as determined by an unpaired two-tailed t-test.

G. Maximum fluorescence values of *pdaf-7::gfp* in the ASJ neurons of adult animals at various time points after being moved from "Fed" to "Bottom" conditions. Each point represents an individual animal, and error bars indicate standard deviation. **** $p < 0.0001$, * $p < 0.05$, ns not significant as determined by an unpaired two-tailed t-test.

H. Maximum fluorescence values of *pdaf-7::gfp* in the ASJ neurons of adult wild type and *del-3(ok2613); del-7(ok1187)* under "Fed," "Bottom," and "Empty" conditions. Each point represents an individual animal, and error bars indicate standard deviation. *** $p < 0.001$, ns not significant as determined by an unpaired two-tailed t-test.

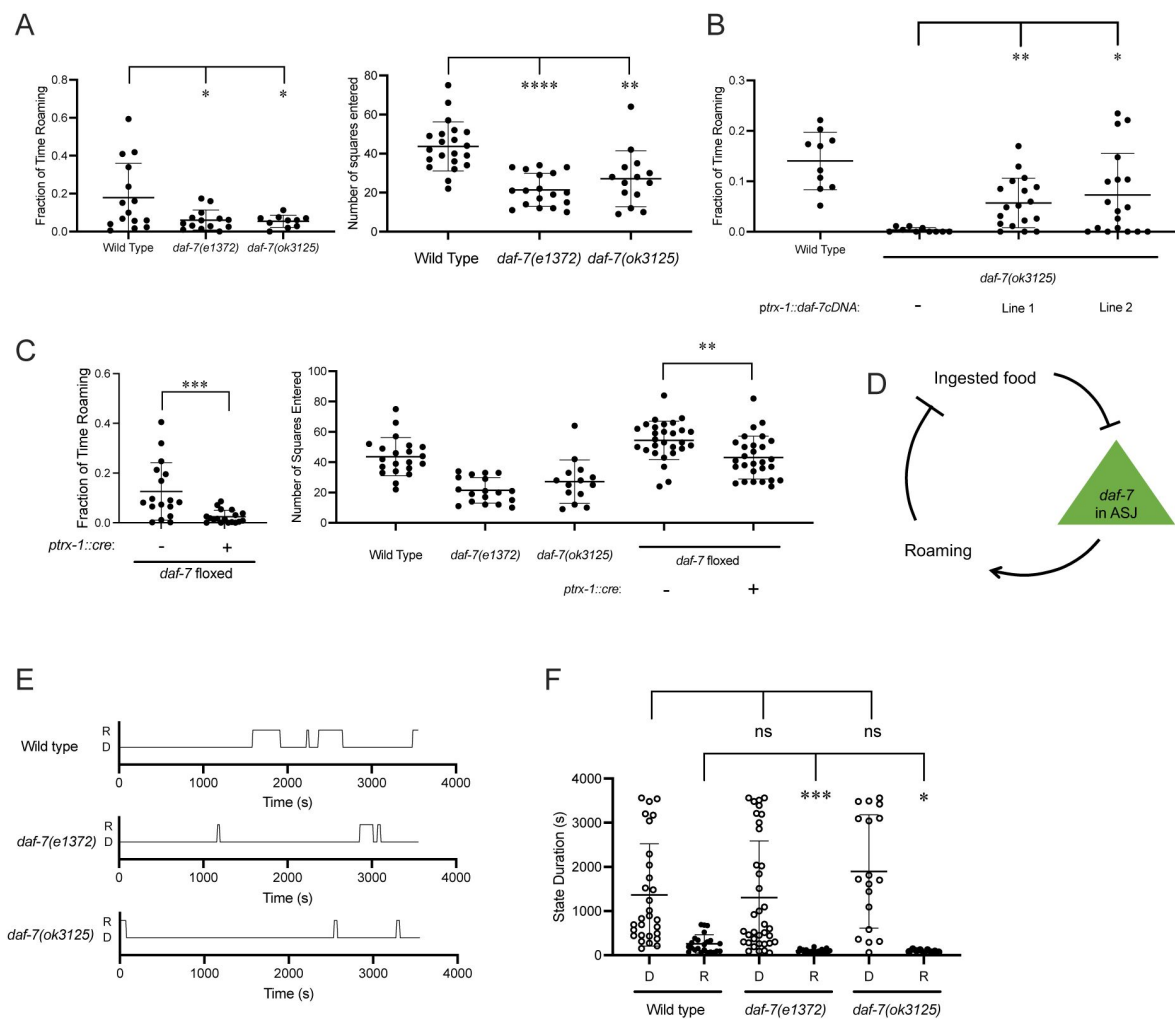


Figure 2.

***daf-7* expression in the ASJ neurons promotes roaming.**

A. Fraction of time roaming (left), and number of squares entered in exploration assay (right) of wild type, *daf-7(e1372)* and *daf-7(ok3125)* animals. Each point represents an individual animal, and error bars indicate standard deviation **** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$ as determined by an unpaired two-tailed t-test.

B. Fraction of time roaming of wild type, *daf-7(ok3125)*, and two independent lines where *daf-7* cDNA was expressed under the *trx-1* promoter in a *daf-7(ok3125)* background. Each point represents an individual animal, and error bars indicate standard deviation. ** $p < 0.01$, * $p < 0.05$ as determined by an unpaired two-tailed t-test.

C. Fraction of time roaming (left), and number of squares entered in exploration assay (right) of wild type, *daf-7(e1372)*, *daf-7(ok3125)*, and a floxed *daf-7* strain with and without Cre expressed under the *trx-1* promoter. Each point represents an individual animal, and error bars indicate standard deviation. ** $p < 0.01$, * $p < 0.05$ as determined by an unpaired two-tailed t-test.

D. Model of positive-feedback relationship between food ingestion, *daf-7* expression in the ASJ neurons, and roaming.

E. Sample trace files of representative individual wild-type, *daf-7(e1372)*, and *daf-7(ok3125)* animals. R = roaming, D= dwelling.

F. Duration of dwelling (open circles) and roaming (closed circles) states for wild type, *daf-7(e1372)* and *daf-7(ok3125)* animals. Each point represents a discrete roaming or dwelling period. Error bars indicate standard deviation. *** $p < 0.001$, * $p < 0.05$, ns not significant as determined by an unpaired two-tailed t-test.

animals are removed from edible food but are still exposed to soluble food signals, upregulation of *daf-7* expression in the ASJ neurons promotes an increased proportion of time in the roaming state. Considering that animals can exist in one of the two states while feeding on bacterial food, a decreased fraction of time roaming could be due to shorter roaming states, longer dwelling states, or combination of these factors. We hypothesized that this transcriptional feedback loop might function in the persistence of internal states underlying roaming and dwelling behaviors, so we measured the duration of roaming and dwelling states in animals lacking DAF-7 signaling.

Representative trace files of wild-type, *daf-7(e1372)*, and *daf-7(ok3125)* animals show how DAF-7 can influence roaming and dwelling state duration (**Figure 2E** [↗](#)). Quantification of state duration across multiple animals revealed that *daf-7* animals exhibited shortened roaming states relative to wild-type animals as predicted by the positive feedback loop (**Figure 2F** [↗](#)). We did not observe a difference in dwelling state duration among the genotypes examined, although we suspect the wide variation dwelling state durations exhibited by animals precluded the detection of a statistically significant difference with our analysis.

Neuromodulatory mechanisms that affect foraging behavior alter *daf-7* expression in the ASJ neurons

The positive-feedback loop supported by our data (**Figure 2D** [↗](#)) suggests a correlation between *daf-7* expression in the ASJ neurons and genetic backgrounds favoring roaming behavior and hence a diminished ingestion of bacterial food. Serotonin has been shown to promote long dwelling states, and thus animals with mutations in *tph-1*, the gene encoding the enzyme that catalyzes the rate-limiting step of serotonin biosynthesis, spend a greater proportion of their time in the roaming state (**Figure 3A** [↗](#)) (Flavell et al., 2013 [↗](#); Ji et al., 2021 [↗](#)). Consistent with the correlation between *daf-7* expression in the ASJ neurons and roaming, we see that *tph-1(n4622)* animals constitutively express *daf-7* in the ASJ neurons on ingestible food, no food, and non-ingestible food, with this *daf-7* expression retaining sensitivity to changes in food environments (**Figure 3B** [↗](#)). These results suggest that serotonin signaling may influence *daf-7* expression in parallel to the gustatory and interoceptive food cues.

Pigment dispersing factor (PDF) signaling has also been previously shown to modulate roaming and dwelling behavior, with animals carrying loss-of-function mutations in the PDF receptor gene *pdf-1* showing a decreased fraction of their time in the roaming state than wild-type animals (**Figure 3C** [↗](#)) (Flavell et al., 2013 [↗](#); Ji et al., 2021 [↗](#)). Consistent with the correlation between roaming and *daf-7* expression in ASJ, we observed that animals with loss-of-function mutations in *pdf-1* do not upregulate *daf-7* transcription in the ASJ neurons on non-ingestible food (**Figure 3D** [↗](#)). From a screen done in parallel to this work, we recovered a putative gain-of-function allele of *pdf-1*, *pdf-1(qd385)*, carrying a S325F substitution (Boor, 2022 [↗](#)). Follow-up analysis in an independently generated S325F allele, *pdf-1(syb3826)*, confirmed that this substitution was responsible for our observed phenotypes. We observed that *pdf-1(syb3826)* animals exhibited increased roaming behavior compared with wild-type animals (**Figure 3C** [↗](#)) and constitutive expression of *daf-7* in the ASJ neurons in the presence of ingestible food, which was further upregulated under conditions of no bacterial food or non-ingestible food (**Figure 3D** [↗](#)). These results are consistent with a model in which PDFR-1 signaling influences *daf-7* expression in the ASJ neurons by modulating the sensation of the external gustatory food cue.

SCD-2 controls *daf-7* expression in the ASJ neurons and roaming

To identify additional genetic factors involved in coupling the ingestion of food to *daf-7* expression in the ASJ neurons and its subsequent effect on roaming, we were guided by the isolation of an allele of *hen-1*, *hen-1(qd259)*, from a screen we previously performed for genes regulating the expression of *daf-7* from the ASJ neurons in response to *P. aeruginosa* (Park et al., 2020 [↗](#)). Through genomic rescue experiments, we confirmed that the *hen-1* mutation was the causative lesion in this strain, and animals with mutations in *scd-2*, encoding the receptor of HEN-1, share

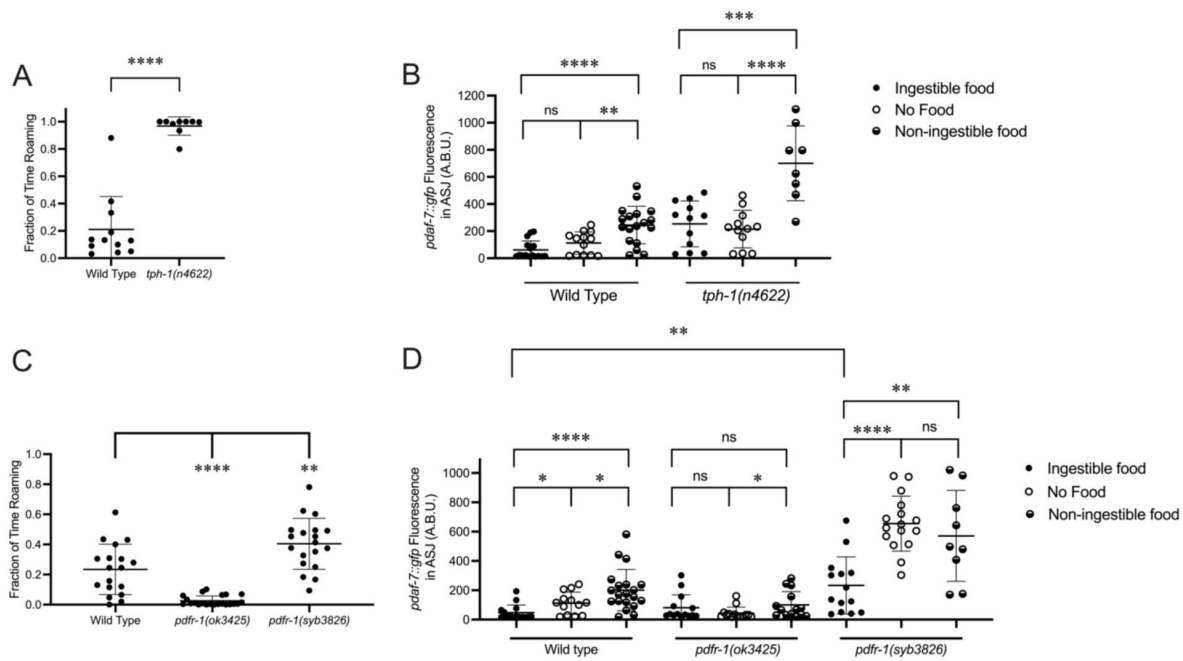


Figure 3.

Known modulators of roaming and dwelling affect *daf-7* expression in the ASJ neurons

A. Fraction of time roaming of wild type and *tph-1(n4622)* animals. Each point represents an individual animal, and error bars indicate standard deviation. **** $p < 0.0001$ as determined by an unpaired two-tailed t-test.

B. Maximum fluorescence values of *pdaf-7::gfp* in the ASJ neurons of adult wild type and *tph-1(n4622)* animals under "Fed," "Empty" and "Bottom" conditions. Each point represents an individual animal, and error bars indicate standard deviation. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, ns not significant as determined by an unpaired two-tailed t-test.

C. Fraction of time roaming of wild type, *pdf-1(ok3425)*, and *pdf-1(syb3826)* animals. Each point represents an individual animal, and error bars indicate standard deviation. **** $p < 0.0001$, ** $p < 0.01$ as determined by an unpaired two-tailed t-test.

D. Maximum fluorescence values of *pdaf-7::gfp* in the ASJ neurons of adult wild type, *pdf-1(ok3425)*, and *pdf-1(syb3826)* animals under "Fed," "Empty," and "Bottom" conditions. Each point represents an individual animal, and error bars indicate standard deviation. **** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$, ns not significant as determined by an unpaired two-tailed t-test.

this phenotype (**Figure 4A** [↗](#)). SCD-2 has characterized for its role in the food-dependent developmental arrest known as dauer diapause and sensory integration (Reiner et al., 2008 [↗](#); Shinkai et al., 2011 [↗](#); Wolfe et al., 2019 [↗](#)), and Anaplastic Lymphoma Kinase (ALK), the human ortholog of SCD-2, has been implicated in metabolic phenotypes in humans, mice, and *Drosophila* (Orthofer et al., 2020 [↗](#)). Taken together, these observations led us to question if SCD-2 might be involved in the regulation of *daf-7* expression in the ASJ neurons in response to changes in food ingestion. We found that animals with loss-of-function alleles of *scd-2* showed reduced upregulation of *daf-7* in the ASJ neurons in response to non-ingestible food compared to wild-type animals, implicating SCD-2 at least partially in this *daf-7* expression response (**Figure 4B** [↗](#)).

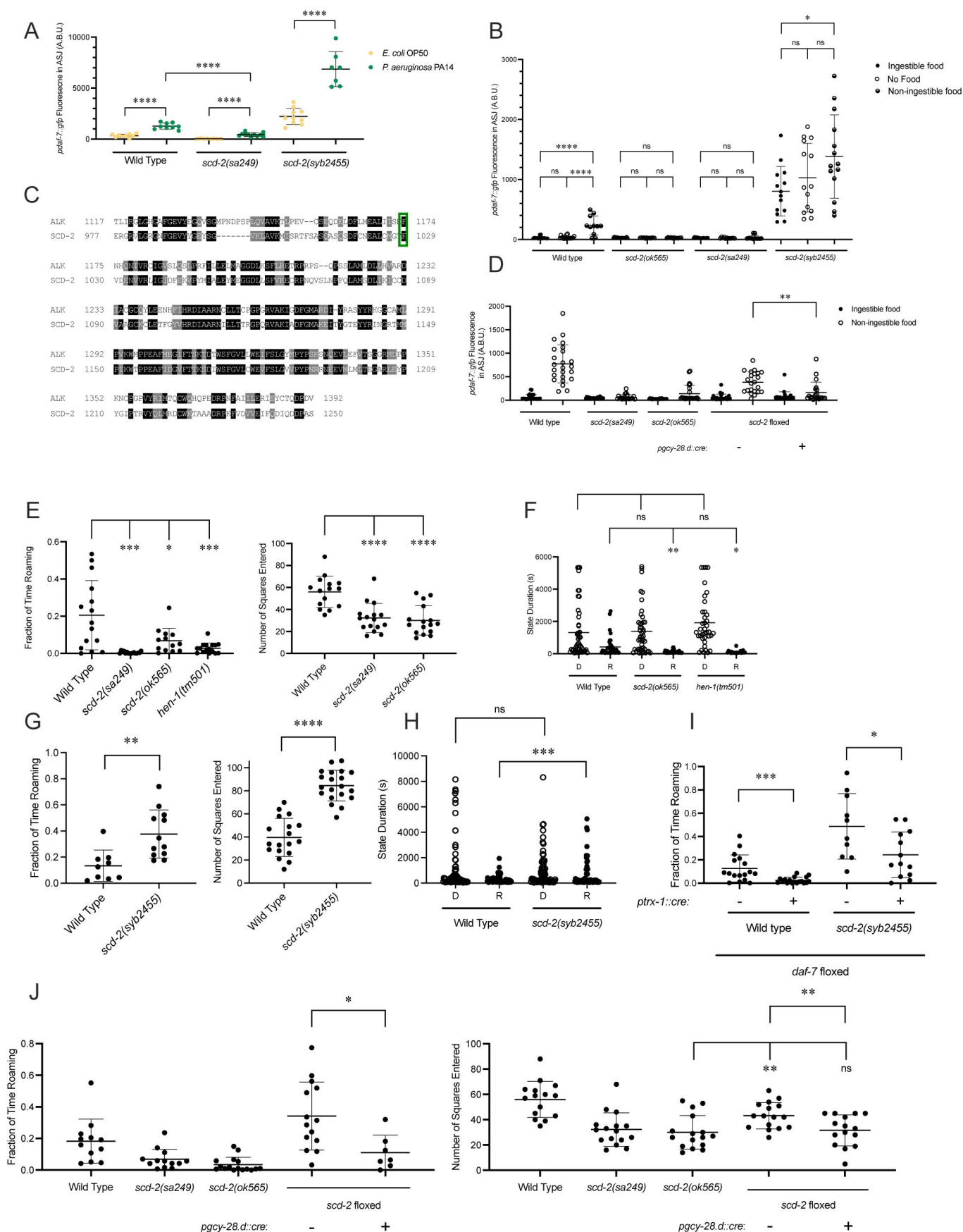


Figure 4.

SCD-2 regulates *daf-7* expression in the ASJ neurons and roaming behavior

- A. Maximum fluorescence values of *pdaf-7::gfp* in the ASJ neurons of adult wild type, *scd-2(sa249)*, and *scd-2(syb2455)* animals exposed to *E. coli* OP50 and *P. aeruginosa* PA14. Each point represents an individual animal, and error bars indicate standard deviation. **** $p < 0.0001$, as determined by an unpaired two-tailed t-test.
- B. Maximum fluorescence values of *pdaf-7::gfp* in the ASJ neurons of adult wild type, *scd-2(ok565)*, *scd-2(sa249)*, and *scd-2(syb2455)* animals under "Fed," "Empty," and "Bottom" conditions. Each point represents an individual animal, and error bars indicate standard deviation. **** $p < 0.0001$, * $p < 0.05$, ns not significant as determined by an unpaired two-tailed t-test. **** $p < 0.0001$, * $p < 0.05$ as determined by an unpaired two-tailed t-test.
- C. Sequence alignment of the kinase domains of human ALK (top) and *C. elegans* SCD-2 (bottom). Amino acids highlighted in black are identical and those in gray are similar. F1174/F1029 is outlined in green.
- D. Maximum fluorescence values of *pdaf-7::gfp* in the ASJ neurons of adult wild type, floxed *scd-2*, *scd-2(sa249)*, *scd-2(ok565)*, and floxed *scd-2* animals with a transgene expressing Cre under the AIA-specific *gcy-28.d* promoter under "Fed" and "Bottom" conditions. Each point represents an individual animal, and error bars indicate standard deviation. ** $p < 0.01$ as determined by an unpaired two-tailed t-test.
- E. Fraction of time spent roaming (left) and number of squares entered in exploration assay (right) for wild type, *scd-2(sa249)*, *scd-2(ok565)* and *hen-1(tm501)* animals. Each point represents an individual animal. Error bars indicate standard deviation. **** $p < 0.0001$, *** $p < 0.001$, * $p < 0.05$ as determined by an unpaired two-tailed t-test.
- F. Duration of roaming (closed circles) and dwelling (open circles) states for wild type, *scd-2(ok565)* and *hen-1(tm501)*. Each point represents a discrete roaming or dwelling period. Error bars indicate standard deviation. ** $p < 0.01$, * $p < 0.05$, ns not significant as determined by an unpaired two-tailed t-test.
- G. Fraction of time spent roaming (left) and number of squares entered in exploration assay (right) for wild type and *scd-2(syb2455)* animals. Each point represents an individual animal. Error bars indicate standard deviation. **** $p < 0.0001$, ** $p < 0.01$ as determined by an unpaired two-tailed t-test.
- H. Duration of roaming (closed circles) and dwelling (open circles) states for wild type and *scd-2(syb2455)* animals. Each point represents a discrete roaming or dwelling period. Error bars indicate standard deviation. *** $p < 0.001$, ns not significant as determined by an unpaired two-tailed t-test.
- I. Fraction of time roaming for wild-type of *scd-2(syb2455)* animals with a floxed allele of *daf-7* with or without an ASJ-specific cre transgene. *** $p < 0.001$, * $p < 0.05$ as determined by an unpaired two-tailed t-test.
- J. Left: Fraction of time spent roaming in worm tracker assay for *scd-2(sa249)*, *scd-2(ok565)* and floxed *scd-2* with or without a transgene expressing Cre under the AIA-specific *gcy-28.d* promoter. Right: number of squares entered in exploration assay for wild type, *scd-2(sa249)*, *scd-2(ok565)*, and floxed *scd-2* with or without a transgene expressing Cre under the AIA-specific *gcy-28.d* promoter. Each point represents an individual animal. Error bars indicate standard deviation. ** $p < 0.01$, * $p < 0.05$, ns not significant as determined by an unpaired two-tailed t-test.

To further examine the role of SCD-2 mediating the *daf-7* expression response to ingested food, we generated a gain-of-function allele. Gain-of-function point mutations in ALK are associated with neuroblastoma and most often occur in the kinase domain, with 85% of all ALK point mutations seen at either F1174 or R1275 (Franco et al., 2013). F1174L, the most common point mutation, results in ALK autophosphorylation and cytokine-independent growth (Chen et al., 2008; George et al., 2008; Hallberg & Palmer, 2013; Holla et al., 2017; Janoueix-Lerosey et al.,

2008 [\[1\]](#)). Analysis of a sequence alignment of SCD-2 and ALK revealed that F1174 in ALK was conserved as F1029 in SCD-2, and we were able to create a gain-of-function allele of *scd-2* by engineering a F1029L substitution (*scd-2(syb2455)*) (**Figure 4C** [\[1\]](#)) (Boor, 2022 [\[1\]](#)). To infer whether SCD-2 activity was sufficient to induce expression of *daf-7* in the ASJ neurons in the absence of ingestible bacterial food, we examined the gain-of-function *scd-2(syb2455)* mutant and observed that these animals constitutively expressed *daf-7* in the ASJ neurons even in the presence of ingestible food (**Figure 4B** [\[1\]](#)). We further observed that the magnitude of upregulation of *daf-7* expression in the ASJ neurons when animals were moved from ingestible food to non-ingestible food was reduced in *scd-2(syb2455)* to levels only about one-fifth of that seen in wild-type animals (the ratio of wild-type *daf-7* expression in the ASJ neurons on non-ingestible food to ingestible food = 8.1; the ratio of *scd-2(syb2455)* *daf-7* expression in the ASJ neurons on non-ingestible food to ingestible food = 1.7) (**Figure 4B** [\[1\]](#)). In contrast to the reduced upregulation seen in the *scd-2(syb2455)* gain-of-function animals when exposed to non-ingestible food, *scd-2(syb2455)* exhibited robust upregulation of *daf-7* expression in the ASJ neurons when exposed to *P. aeruginosa* (**Figure 4A** [\[1\]](#)). The observation that this PA14-dependent upregulation is intact in *scd-2(syb2455)* animals while the non-ingestible food-dependent upregulation is attenuated supports a direct role for SCD-2 in the response to ingested food rather than a global control of *daf-7* expression in the ASJ neurons.

Expression of *scd-2* cDNA under the AIA-specific *gcy-28.d* promoter has been shown to be sufficient to rescue behavioral phenotypes of *scd-2* mutants that have been attributed to a role for AIA-expressed SCD-2 in sensory integration (Shinkai et al., 2011 [\[1\]](#); Wolfe et al., 2019 [\[1\]](#)). To ask whether SCD-2 could be functioning in the AIA neurons to regulate *daf-7* expression in the ASJ neurons, we generated a strain with the coding sequence of SCD-2 floxed and introduced a transgene expressing Cre under the *gcy-28.d* promoter. Compared to animals containing a floxed *scd-2* allele without Cre, animals with AIA-specific Cre expression resulting in AIA-specific deletion of *scd-2* showed reduced upregulation of *daf-7* expression in the ASJ neurons in response to non-ingestible food, suggesting that SCD-2 activity in the AIA neurons acts cell-non-autonomously to control *daf-7* expression in the ASJ neurons (**Figure 4D** [\[1\]](#)).

Our finding that SCD-2 regulates the *daf-7* expression in response to ingested food predicts that SCD-2 would also promote roaming behavior. Consistent with this hypothesis, we observed that animals with loss-of-function mutations in *scd-2* spent a lower proportion of time roaming and explored less of the lawn in an exploration assay than wild-type animals (**Figure 4E** [\[1\]](#)). Animals with a loss-of-function allele of *hen-1* also roamed less than wild type (**Figure 4E** [\[1\]](#)). Furthermore, as predicted by the regulation of *daf-7* expression in the ASJ neurons, *scd-2* and *hen-1* animals exhibit shorter roaming states and unchanged dwelling state durations compared to wild-type animals (**Figure 4F** [\[1\]](#)). When we examined the roaming and dwelling behavior of *scd-2(syb2455)* gain-of-function animals, we found that these mutants showed increased roaming behavior and lawn exploration as compared to wild-type animals and exhibited longer roaming states (**Figure 4G** [\[1\]](#) and **4H** [\[1\]](#)). Consistent with SCD-2 mediating roaming partially through its influence on *daf-7* expression in the ASJ neurons, we saw that the increased roaming exhibited by *scd-2(syb2455)* animals was reduced in animals with a Cre-lox-mediated ASJ-specific *daf-7* deletion (**Figure 4I** [\[1\]](#)). As with the regulation of *daf-7* expression in the ASJ neurons, we observed that Cre-lox-mediated deletion of *scd-2* specifically in the AIA neurons reduced both roaming and exploration compared to floxed *scd-2* animals without Cre, suggesting that SCD-2 functions in the AIA interneurons to promote roaming behavior (**Figure 4J** [\[1\]](#)).

A neuronal gene expression correlate of internal state dynamics driving foraging behavior

Considering our positive feedback model, we reasoned that conditions under which we have observed *daf-7* expression in the ASJ neurons would correlate with increased roaming behavior. Previous work from our lab has shown that upon reaching reproductive maturity, male *C. elegans*

induce *daf-7* expression in their ASJ neurons, which contributes to male mate-searching behavior (Figure 5A) (Hilbert & Kim, 2017). Consistent with the behaviors that have been reported for males and hermaphrodites in bacterial food-leaving assays (Lipton et al., 2004), we observed that wild-type males spend a greater proportion of their time in the roaming state than hermaphrodites (Figure 5B). We also observed that the *daf-7* expression in the ASJ neurons in males was sensitive to the inhibitory effects of ingested food, although the magnitude of the fold increase in *daf-7* expression from the ASJ neurons was about half that observed for hermaphrodites (Figure 5A).

We have also previously reported an upregulation in *daf-7* expression in the ASJ neurons when animals are on pathogenic *P. aeruginosa* PA14 in response to specific secondary metabolites phenazine-1-carboxamide and pyochelin (Figure 5C) (Meisel et al., 2014). Analysis of roaming and dwelling on PA14 revealed that animals significantly increase the fraction of their time in the roaming state on PA14 compared to nonpathogenic *E. coli* OP50 (Figure 5D). We observed that upon exposure to the nonpathogenic PA14 mutant Δ gacA, which results in an intermediate level of *daf-7* expression in the ASJ neurons (Figure 5C) (Meisel et al., 2014), animals roamed an intermediate amount between animals on OP50 and pathogenic PA14 (Figure 5D).

Discussion

A neuroendocrine gene expression feedback loop couples the ingestion of bacterial food to foraging behavior

The data we present here support a model in which bacterial food ingestion regulates roaming and dwelling behavior in part via SCD-2-dependent *daf-7* expression in the ASJ neurons. Our identification of the AIA neurons as the site of SCD-2 action is consistent with a role for the AIA neurons in relaying food signals in other contexts. AIA has been characterized as a downstream synaptic target of multiple food sensory neurons to integrate information about the food environment and influence chemoreceptor expression in other neurons (Dobosiewicz et al., 2019; McLachlan et al., 2022).

We have observed that the expression of *daf-7* in the ASJ neurons responds to two opposing food cues. An interoceptive cue derived from the sensation of ingested food in the pharynx inhibits the expression of *daf-7* in the ASJ neurons, while the presence of a diffusible non-ingested food cue induces the expression of *daf-7* in the ASJ neurons (Figure 1B). In the presence of both food cues, as on abundant ingestible food, the interoceptive food signal is epistatic to the external food signal, promoting exploitation of the current food environment with low levels of *daf-7* expression in the ASJ neurons. Our identification of two opposing food cues is consistent with findings from Wexler et al., which reported that aztreonam-treated bacteria could restore *daf-7* expression in the ASJ neurons of starved males, indicative of an external food cue that is upregulating *daf-7* expression in the ASJ neurons (Wexler et al., 2020). Furthermore, the activity of the ASJ neurons have been demonstrated to be modulated by removal of bacterial supernatant (Zaslaver et al., 2015), consistent with ASJ responsiveness to an external food cue.

As discussed above, the relationship between foraging behavior, food ingestion, and SCD-2-dependent *daf-7* expression has led us to posit a positive-feedback loop that couples food ingestion with foraging behavior (Figure 2D). As animals in a patchy food environment encounter a region with less edible food, our model predicts that a reduction of ingested food would result in *daf-7* expression induction in the ASJ neurons via SCD-2. This increase in *daf-7* expression is correlated with an internal state that favors roaming. As animals roam, they ingest less bacteria, further promoting SCD-2-dependent *daf-7* expression in the ASJ neurons, stabilizing a roaming-prone state. Once a roaming animal encounters edible food and begins to eat, the interoceptive sensation of food in the pharynx results in a downregulation of *daf-7* expression in the ASJ

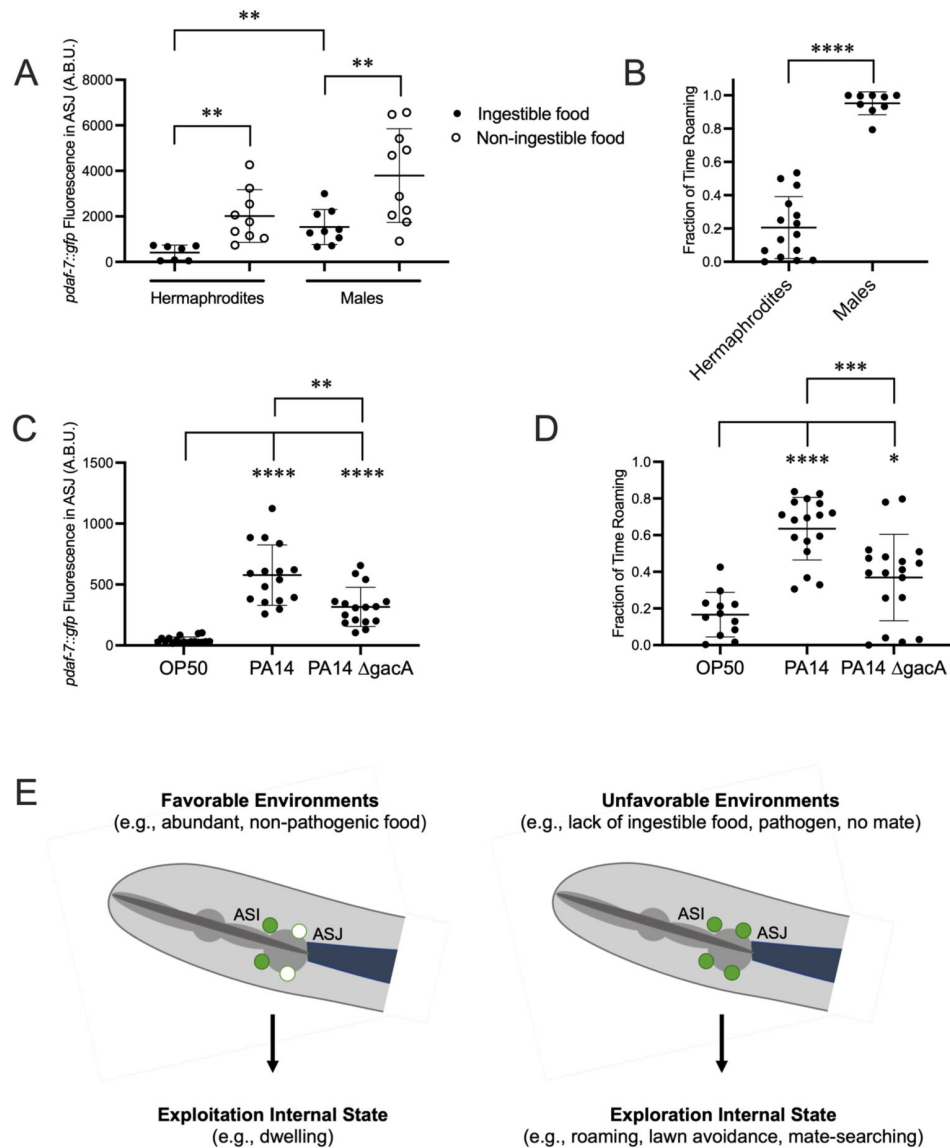


Figure 5.

DAF-7 expression in the ASJ neurons correlates with an increase in roaming behavior under various conditions

A. Maximum fluorescence values of *pdaf-7::gfp* in the ASJ neurons of adult hermaphrodites and males under “Fed” and “Aztreonam” conditions. Each point represents an individual animal, and error bars indicate standard deviation. ** $p < 0.01$ as determined by an unpaired t-test.

B. Fraction of time roaming of wild-type hermaphrodites and males. Each point represents an individual animal, and error bars indicate standard deviation. **** $p < 0.0001$ as determined by an unpaired t-test.

C. Maximum fluorescence values of *pdaf-7::gfp* in the ASJ neurons of adult hermaphrodites fed *E. coli* OP50, *P. aeruginosa* PA14 or *P. aeruginosa* PA14ΔgacA. Each point represents an individual animal, and error bars indicate standard deviation. **** $p < 0.0001$, ** $p < 0.01$ as determined by an unpaired t-test.

D. Fraction of time roaming of wild-type hermaphrodites on *E. coli* OP50, *P. aeruginosa* PA14, or PA14 ΔgacA. Each point represents an individual animal, and error bars indicate standard deviation. **** $p < 0.0001$, *** $p < 0.001$, * $p < 0.05$ as determined by an unpaired t-test.

E. *daf-7* expression in the ASJ neurons responds to environmental conditions and is correlated with internal state. Under favorable conditions, *daf-7* is not expressed in the ASJ neurons, and this is correlated with an internal state that favors exploitation of the animal’s current environment. Under unfavorable conditions, *daf-7* expression is induced in the ASJ neurons, consistent with an internal state that favors exploration.

neurons, promoting a dwelling state in which the animal can continue to eat. As additional food is ingested, this further reduces *daf-7* expression in the ASJ neurons, stabilizing this internal state that favors dwelling. Moreover, a recent study has highlighted the role of the ASJ neurons in promoting roaming and food-leaving behavior (Scheer & Bargmann, 2023 [DOI](#)). We additionally find that PDF and serotonin signaling appear to contribute to *daf-7* expression in the ASJ neurons in addition to their previously characterized roles in roaming and dwelling (Flavell et al., 2013 [DOI](#); Ji et al., 2021 [DOI](#)).

A number of recent studies in *C. elegans* point to a key role for neuronal transcriptional responses in the regulation of behavioral plasticity in response to changing environmental stimuli such as food and temperature (Harris et al., 2023 [DOI](#); Kyani-Rogers et al., 2022 [DOI](#); Ryan et al., 2014 [DOI](#); Wexler et al., 2020 [DOI](#)). For example, differential expression of the diacetyl-sensing chemoreceptor ODR-10 in the AWA neurons in response to changing food conditions, which functions downstream of *daf-7* expression in the ASJ neurons of males, has been shown to modulate food-leaving mate-searching behavior (Ryan et al., 2014 [DOI](#); Wexler et al., 2020 [DOI](#)). We anticipate that the characterization of neuron-specific transcriptional changes that are correlated with *daf-7* expression in the ASJ neurons may yield additional mechanistic insights into how changing environmental and endogenous ingested bacterial food levels modulate internal states driving foraging behavior.

SCD-2/ALK controls a neuroendocrine response to the ingestion of bacterial food

Here, we show that *daf-7* expression in the ASJ neurons is regulated by the receptor tyrosine kinase SCD-2. SCD-2 and its ligand, HEN-1, have been implicated sensory integration, associative learning, and memory (Ishihara et al., 2002 [DOI](#); Shinkai et al., 2011 [DOI](#); Wolfe et al., 2019 [DOI](#)), and SCD-2 was initially characterized for its role in regulating dauer formation, a developmentally arrested state induced by stressful growth conditions, including lack of nutritious food (Golden & Riddle, 1982 [DOI](#), 1984a [DOI](#), 1984b [DOI](#), 1984c [DOI](#); Inoue & Thomas, 2000 [DOI](#); Reiner et al., 2008 [DOI](#)). SCD-2 is the *C. elegans* ortholog of Anaplastic Lymphoma Kinase (ALK). In humans, ALK is expressed primarily in the nervous system and influences cell proliferation, differentiation, and survival in response to external stimuli (Iwahara et al., 1997 [DOI](#)). Genetic translocations resulting in gene fusions are common in ALK-dependent human cancers, including non-small cell lung cancer (NSCLC), diffuse large B cell lymphoma (DLBCL), squamous cell carcinoma (SCC), and renal cell carcinoma (RCC) (Hallberg & Palmer, 2013 [DOI](#); Holla et al., 2017 [DOI](#); Morris et al., 1994 [DOI](#)). Recent studies have suggested a physiological role for ALK in the neuronal control of metabolism. A genome-wide association study (GWAS) looking for genetic variants associated with thinness identified a variant in the first intron of ALK (Orthofer et al., 2020 [DOI](#)). Additional studies in *Drosophila* and mice have further implicated ALK in triglyceride accumulation, starvation survival, and metabolic response to high-fat diets (Cheng et al., 2011 [DOI](#); Orthofer et al., 2020 [DOI](#); Woodling et al., 2020 [DOI](#)).

We have observed a role for SCD-2 in regulating foraging behavior and neuroendocrine gene expression in response to changing food conditions. We and others have observed that animals with loss-of-function mutations in *scd-2* behave as if they are in the constant presence of ingested “good” food; *scd-2* animals dwell more, reduce upregulation of *daf-7* expression in the ASJ neurons in the absence of ingested food, and fail to enter dauer under poor food conditions (Reiner et al., 2008 [DOI](#)). In contrast, animals with the gain-of-function *scd-2(syb2455)* allele act like animals exposed to “bad” food conditions, as evidenced by increased roaming, constitutive expression of *daf-7* in their ASJ neurons, and food-independent constitutive dauer entry (Boor, 2022 [DOI](#)). If SCD-2 is functioning in responding to changes in food, this may alter conclusions of several previous studies implicating HEN-1 and SCD-2 in sensory integration, as several of the assays employed in these studies have been shown to be affected by the animals’ nutritional status (Ishihara et al., 2002 [DOI](#); Shinkai et al., 2011 [DOI](#); Wolfe et al., 2019 [DOI](#)). Further investigation into the role of SCD-2 in responding to food vs. sensory integration in these assays could be informative.

Furthermore, the role for SCD-2 in regulating the physiological response to ingested food in *C. elegans* is consistent with the growing body of work tying ALK and its orthologs to thinness and metabolic phenotypes in humans, mice, and *Drosophila* (Cheng et al., 2011 [DOI](#); Orthofer et al., 2020 [DOI](#); Woodling et al., 2020 [DOI](#)). Our observations of *scd-2* *C. elegans* are consistent with phenotypes seen in *ALK*^{-/-} mice and *Alk* RNAi *Drosophila*, including increased energy expenditure and reduced triglyceride accumulation (Orthofer et al., 2020 [DOI](#)). Since the phenotypes of *scd-2* animals are associated with abundant food in *C. elegans*, we might expect these *ALK*^{-/-} mice and *Alk* RNAi *Drosophila* to mimic animals that are constantly eating and engage in compensatory mechanisms of increased energy expenditure and triglyceride metabolism. Further characterization of the mechanisms by which SCD-2 and ALK regulate food-dependent behavior, gene expression, and metabolism could reveal new factors governing body weight and have implications in the treatment and prevention of obesity.

***daf-7* expression in the ASJ neurons is correlated with an internal state favoring exploration**

The response of *daf-7* expression in the ASJ neurons to changes in food conditions and its effect on foraging behavior provides an example of the influence of gene expression on internal states. We have identified a transcriptional switch of one gene in two cells that can influence foraging behavior in a manner as equally simple as but on a longer time scale than neuronal firing (Figure 5E [DOI](#)). This *daf-7*-expressing, pro-roaming internal state might drive other exploratory behaviors that our lab and others have observed under conditions where *daf-7* is expressed in the ASJ neurons. Male *C. elegans* have been observed to upregulate the expression of *daf-7* in their ASJ neurons upon reaching reproductive maturity, and this *daf-7* expression has been implicated in driving mate-searching behavior (Hilbert & Kim, 2017 [DOI](#)). Males must find a hermaphrodite mate to reproduce – a requirement not shared by hermaphrodites – and they often must leave food to do so. The constitutive expression of *daf-7* in the ASJ neurons of adult males could be due to a reduced sensitivity to ingested food allowing for prioritization of searching for a mate over feeding. This is consistent with our observation that *daf-7* expression in the ASJ neurons is upregulated less in response to non-ingestible food in males than in hermaphrodites.

Similarly, our lab has previously reported that two secondary metabolites of pathogenic *P. aeruginosa* can result in the upregulation of *daf-7* expression in the ASJ neurons, which contributes to pathogen avoidance behavior (Meisel et al., 2014 [DOI](#)). The results presented here suggest that perhaps these secondary metabolites are tapping into this foraging circuit and overriding other inputs that regulate *daf-7* expression in the ASJ neurons to promote the animals moving away from the pathogen in a manner like roaming animals seeking nutritious food. In the natural environment of *C. elegans*, pathogenic and nutritious bacteria coexist, and animals must employ mechanisms to avoid infection while still obtaining adequate nutrition. Low levels of nutritious food, the presence of pathogen, or an absence of mates each represents a suboptimal environment and may induce a common internal state that promotes exploration and enhances the chances of encountering more favorable conditions.

Multiple integrative neuronal mechanisms likely converge to establish “internal state” that modulates organism behavior. Our data reveal that gene expression, specifically the expression of a single gene, *daf-7*, from just two neurons, the ASJ chemosensory neurons, can not only contribute to internal state driving foraging behavior through its role in a neuroendocrine feedback loop, but also serve as a readily detected correlate of internal state underlying foraging behavior.

Materials and Methods

C. elegans strains

C. elegans was maintained on *E. coli* OP50 as previously described (Brenner, 1974). Daf-c strains were grown at 16°C. See **Table 1** for a complete list of strains used in this study.

Design of potential *scd-2* gain-of-function alleles

We performed a protein alignment of *C. elegans* SCD-2 and human ALK in NCBI BLAST (**Figure 4C**). Using a list of known oncogenic ALK mutations (Holla et al., 2017), we screened these residues for conservation or similarity between the *C. elegans* and human protein sequences. Genome editing was done by SunyBiotech using CRISPR technology. Alleles were evaluated in a dauer assay for a gain-of-function Daf-c phenotype, and validated in trans-heterozygote analysis with *scd-2(sa249)* for a dominant phenotype (Boor, 2022).

Preparation of food condition plates

Unless otherwise indicated, all assays were performed on NGM plates with no peptone. Aztreonam-treated bacteria was prepared as previously reported (Gruninger et al., 2008), and 40 µL aztreonam-treated food was added to plates containing 10 µg/mL aztreonam. “Fed” plates for *daf-7* quantification were seeded with 40 µL OP50 grown overnight in a shaking LB culture at 37°C. “Bottom” plates were seeded with 250 µL OP50, and the agar was inverted with a spatula immediately prior to adding animals. “Lid” plates were prepared by pouring a spot of NGM agar on the inside of the lid of the plate and seeding 100 µL of OP50 on to the spot. PA14 was grown as previously described and seeded onto SKA plates, as were *E. coli* OP50 controls for these experiments (Meisel et al., 2014).

pdaf-7::gfp quantification assays

Plates of gravid animals were bleached and eggs were dropped onto NGM plates seeded with OP50 and grown at 20°C for 67 hours, unless otherwise noted. On the day of imaging, 15–30 day one adult animals were transferred by picking to assay plates, where they were incubated for 5 hours at 20°C. Animals to be imaged were mounted on glass slides with agarose pads and 50 mM sodium azide or 5 mM Levamisole. All imaging for pictures were conducted on the Zeiss Axioimager Z1. Quantification of GFP brightness was derived from maximum fluorescence values within the ASJ neurons in FIJI.

Exploration assay

The exploration assay was performed loosely as previously described with several modifications (Flavell et al., 2013). Thirty-five millimeter NGM plates with no peptone were seeded with 500 µL OP50 grown overnight in LB so that the lawn covered the entire surface of the plate. On the day of the assay, one day-one adult animal was placed on the plate and allowed to explore for two hours before being removed. The plate was then superimposed on a grid of 3.5 mm squares and the number of squares crossed by the *C. elegans* tracks was manually counted.

Roaming/dwelling assay

Animals were egg-laid and grown to day-one adults. 10 cm NGM plates without peptone were seeded with 2 mL stationary phase *E. coli* OP50 grown overnight in LB. Assays were performed with ~20 animals inside a 6 cm copper ring placed in the center of the seeded plate. After one hour for the animals to adjust to their new environment, videos were recorded at 3.75 frames per second for 1.5–3 hours. Videos were analyzed using MBF Biosciences WormLab software (WormLab, 2020).

Strain Name	Genotype	Source
N2	Wild type	Caenorhabditis Genetics Center (CGC)
JT249	<i>scd-2(sa249)</i>	CGC
RB783	<i>scd-2(ok565)</i>	CGC
JC2154	<i>hen-1(tm501)</i>	CGC
PHX2455	<i>scd-2(syb2455)</i>	This study/SunyBiotech
FK181	<i>ksIs2[pdaf-7::gfp; rol-6(su1006)]</i>	CGC
ZD2540	<i>ksIs2; scd-2(sa249)</i>	This study
ZD930	<i>ksIs2; scd-2(ok565)</i>	This study
ZD918	<i>ksIs2; hen-1(tm501)</i>	This study
ZD2605	<i>ksIs2; scd-2(syb2455)</i>	This study
CB1372	<i>daf-7(e1372)</i>	CGC
ZD715	<i>daf-7(ok3125)</i>	(Meisel et al., 2014)
ZD695	<i>daf-7(ok3125); qdEx34[ptrx-1::daf-7; pges-1::GFP]</i>	(Meisel et al., 2014)
ZD696	<i>daf-7(ok3125); qdEx35[ptrx-1::daf-7; pges-1::GFP]</i>	(Meisel et al., 2014)
ZD2632	<i>ksIs2; del-3(ok2613); del-7(ok1187)</i>	This study
MT14984	<i>tph-1(n4622)</i>	Horvitz Lab
ZD667	<i>ksIs2; tph-1(n4622)</i>	This study
ZD2079	<i>pdf-1(ok3425)</i>	(Hilbert & Kim, 2018)
PHX3826	<i>pdf-1(syb3826)</i>	This study/SunyBiotech
ZD1987	<i>ksIs2; pdf-1(ok3425); him-5(e1490)</i>	(Hilbert & Kim, 2018)
ZD2633	<i>ksIs2; pdf-1(syb3826); him-5(e1490)</i>	This study
ZD2721	<i>scd-2(syb5845 syb6052) Backcrossed x2</i>	This study
ZD2722	<i>daf-7(syb5855 syb5965) qdEx[ptrx-1::Cre; pofm-1::gfp] Backcrossed x2</i>	This study
ZD2752	<i>scd-2(syb5845 syb6052); ex[gcy-28.dp::cre; ofm-1p::gfp]</i>	This study
ZD2766	<i>scd-2(syb5845 syb6052); ex[gcy-28.dp::cre; ofm-1p::gfp]; ksIs2</i>	This study
ZD1005	<i>him-5(e1390); ksIs2</i>	(Hilbert & Kim, 2017)

Table 1.

Complete list of *C. elegans* strains used in this study.

Measurements of speed and bending angle (midpoint) were averaged over 10 second intervals, and values for each 10-second interval were plotted on a scatter plot of speed ($\mu\text{m}/\text{sec}$) vs. bending angle (degrees). Quantification of fraction of time spent roaming or dwelling was done by segregating the points of the scatter plots by a horizontal line whose placement was based on the distribution of points in the control condition for each experiment. Values for this speed cutoff ranged from $y=7$ to $y=12$. Points falling above the line were classified as roaming, and those below the line were classified as dwelling. The fraction of time spent roaming was calculated for each animal based off the speed cutoff. Outliers were removed using a ROUT test with $Q = 1\%$. The durations of roaming and dwelling states were determined manually by analyzing average speed values for successive ten-second intervals. Only states lasting longer than five intervals (fifty seconds) were considered.

Statistics

All statistical analysis was performed using the GraphPad Prism software (Graphpad Prism, RRID: SCR_002798). Statistical tests used are indicated in each figure legend.

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Reviewer #1 (Public Review):

Summary:

Here, Boor et al focus on the regulation of daf-7 transcription in the ASJ chemosensory neurons, which has previously shown to be sensitive to a variety of external and internal signals. Interestingly, they find that soluble (but not volatile) signals released by food activate daf-7 expression in ASJ, but that this is counteracted by signals from the ASIC channels del-3 and del-7, previously shown to detect the ingestion of food in the pharynx. Importantly, the authors find that ASJ-derived daf-7 can promote exploration, suggesting a feedback loop that influences locomotor states to promote feeding behavior. They also implicate signals known to regulate exploratory behavior (the neuropeptide receptor PDFR-1 and the neuromodulator serotonin) in the regulation of daf-7 expression in ASJ. Additionally, they identify a novel role for a pathway previously implicated in *C. elegans* sensory behavior, HEN-1/SCD-2, in the regulation of daf-7 in ASJ, suggesting that the SCD-2 homolog ALK may have a conserved role in feeding and metabolism.

Strengths:

The studies reported here, particularly the quantitation of gene expression and the careful behavioral analysis, are rigorously done and interpreted appropriately. The results suggest that, with respect to food, DAF-7 expression encodes a state of "unmet need" - the availability of nearby food to animals that are not currently eating. This is an interesting finding that reinforces and extends our understanding of the neurobiological significance of this important signaling pathway. The identification of a role for ASJ-derived daf-7 in motor behavior is a valuable advance, as is the finding that SCD-2 acts in the AIA interneurons to influence daf-7 expression in ASJ.

Weaknesses:

A limitation of the work is that some mechanistic relationships between the identified signaling pathways remains unclear, but this provides interesting opportunities for future work. There are some minor concerns about the statistical analysis in the paper, but these are unlikely to affect the authors' interpretation of their results.

- <https://doi.org/10.7554/eLife.91120.2.sa2>

Reviewer #2 (Public Review):

In this work, Boor and colleagues explored the role of microbial food cues in the regulation of neuroendocrine controlled foraging behavior. Consistent with previous reports, the authors find that *C. elegans* foraging behavior is regulated by the neuroendocrine TGF β ligand encoded by daf-7. In addition to its known role in the neuroendocrine/sensory ASI neurons,

Boor and colleagues show that *daf-7* expression is dynamically regulated in the ASJ sensory neurons by microbial food cues - and that this regulation is important for exploration/exploitation balance during foraging. They identify at least two independent pathways by which microbial cues regulate *daf-7* expression in ASJ: a gustatory pathway that promotes *daf-7* expression and an opposing interoceptive pathway, also chemosensory in nature but which requires microbial ingestion to inhibit *daf-7* expression via ASIC channels, encoded by *del-3/del-7*. In contrast, the authors show that the conserved PDF neuropeptide signaling pathway likely functions via the gustatory pathway to promote *daf-7* expression. They further identify a novel role for the *C. elegans* ALK orthologue encoded by *scd-2*, which acts in interneurons to regulate *daf-7* expression and foraging behavior. These results together imply that distinct cues from microbial food are used to regulate the balance between exploration and exploitation via conserved signaling pathways.

Strengths:

The findings that gustatory and interoceptive inputs into foraging behavior are separable and opposing are novel and interesting, which they have shown most clearly in Figure 1 and Figure 3. These data clarify how these parallel chemosensory pathways can be integrated at the level of *daf-7* expression.

It is also clear from their results that removal of the interoceptive cue (via transfer to non-digestible food) results in rapid induction of *daf-7::gfp* in ASJ - suggesting that this pathway is likely chemosensory and not simply nutritive in nature. They have also shown that *daf-7* in ASJ plays an important role in the regulation of foraging behavior.

The role of the *hen-1/scd-2* pathway in mediating the effects of ingested food is also compelling and well-interpreted, with a few small caveats, described below. This implies that important elements of this food sensing pathway may be conserved in mammals.

Weaknesses:

Although not a weakness of this work per se, the roles of the 5-HT and *hen-1/scd-2* pathway remain a bit unclear, likely reflecting their complex genetic contributions to foraging and *daf-7* expression. Future work should clarify how these signals are integrated and whether the integration of these pathways improve exploration/exploitation balance to regulate animal fitness.

- <https://doi.org/10.7554/eLife.91120.2.sa1>

Reviewer #3 (Public Review):

Summary:

In this interesting study, the authors characterize the mechanisms whereby a *C. elegans* TGF-beta DAF-7 responds to various forms of food cues to regulate foraging.

Building on their previous findings that characterized the functional role of *daf-7* in the ASJ sensory neurons in response to a bacterial pathogen and in regulating searching behaviors, the authors of this manuscript show that ingestion of *E. coli* OP50, a common laboratory food for the worms, suppresses ASJ expression of *daf-7* and secreted water-soluble cues of OP50 increase it. They further show that the level of *daf-7* expression in ASJ is positively associated with a higher level of roaming/exploration. The authors identify that the function of a *C. elegans* ortholog of Anaplastic Lymphoma Kinase in the interneurons AIA regulates ASJ expression of *daf-7* in response to food information and the related searching behavior.

Strengths:

The study addresses an important question that appeals to a wide readership. The findings are demonstrated by strong results produced from well designed experiments.

- <https://doi.org/10.7554/eLife.91120.2.sa0>

Author Response

The following is the authors' response to the original reviews.

Public Reviews:

Reviewer #1 (Public Review):

Summary:

Here, Boor et al focus on the regulation of daf-7 transcription in the ASJ chemosensory neurons, which has previously been shown to be sensitive to a variety of external and internal signals. Interestingly, they find that soluble (but not volatile) signals released by food activate daf-7 expression in ASJ, but that this is counteracted by signals from the ASIC channels del-3 and del-7, previously shown to detect the ingestion of food in the pharynx. Importantly, the authors find that ASJ-derived daf-7 can promote exploration, suggesting a feedback loop that influences locomotor states to promote feeding behavior. They also implicate signals known to regulate exploratory behavior (the neuropeptide receptor PDFR-1 and the neuromodulator serotonin) in the regulation of daf-7 expression in ASJ. Additionally, they identify a novel role for a pathway previously implicated in C. elegans sensory behavior, HEN1/SCD-2, in the regulation of daf-7 in ASJ, suggesting that the SCD-2 homolog ALK may have a conserved role in feeding and metabolism.

Strengths:

The studies reported here, particularly the quantitation of gene expression and the careful behavioral analysis, are rigorously done and interpreted appropriately. The results suggest that, with respect to food, DAF-7 expression encodes a state of "unmet need" - the availability of nearby food to animals that are not currently eating. This is an interesting finding that reinforces and extends our understanding of the neurobiological significance of this important signaling pathway. The identification of a role for ASJ-derived daf-7 in motor behavior is a valuable advance, as is the finding that SCD-2 acts in the AIA interneurons to influence daf-7 expression in ASJ.

We appreciate the Reviewer 1's thoughtful assessment of our work and inference that the expression of daf-7 encodes internal state corresponding to "unmet need." Based on comments of Reviewer 1 and other reviewers, we have revised the title, abstract, and parts of the discussion to highlight not only the functional contribution of daf-7 expression in the ASJ neurons to behavioral state, but also the remarkable correlation between gene expression and internal state driving foraging behavior.

Weaknesses:

A limitation of the work is that some mechanistic relationships between the identified signaling pathways are not carefully examined, but this provides interesting opportunities for future work.

To enable the reader to begin to infer the relative contributions of the identified signaling pathways to the circuitry coupling distinct bacterial cues to foraging behavior, we have added data for the analysis of DAF-7 expression in the ASJ neurons in the *tph-1* and *pdf-1* mutants in the complete absence of food. Our current leaning is that multiple pathways, including those we have begun to characterize here, may function in parallel to influence DAF-7

expression and internal state driving foraging behavior. Future work to explore this further is certainly of interest.

A minor weakness concerns the experiment in which daf-7 is conditionally deleted from ASJ. This is an ideal approach for probing the function of daf-7, but these experiments seem to be carried out in the well-fed, on-food condition in which control animals should express little or no daf-7 in ASJ. Thus, the experimental design does not allow an assessment of the role of daf-7 under conditions in which its expression is activated (e.g., in animals exposed to un-ingestible food).

The interpretation of genetic analysis in the complete absence of food is complicated by what we think are multiple parallel pathways that function to strongly promote roaming, as indicated in the prior work of Ben Arous et al. Our observation that the conditional deletion of daf-7 from the ASJ pair of neurons confers altered roaming behavior on a lawn of bacterial food supports physiological ongoing role for dynamic daf-7 expression from the ASJ neurons even in the presence of bacterial food that may contribute to the control of transitions between foraging states and the persistence of roaming and dwelling states.

To demonstrate the functional contribution of DAF-7 expression from the ASJ neuron pair during constitutive expression favoring roaming, we examined the roaming behavior of *scd2(syb2455)* animals that carry a gain-of-function mutation in *scd-2* that promotes roaming and how the selective deletion of daf-7 from the ASJ neurons in the *scd-2(syb2455)* genetic background influences roaming behavior. This new experiment supports a model in which DAF-7 expression from the ASJ neurons contributes to the increased roaming behavior exhibited by *scd-2(syb2455)* animals. The new experiment is added as Figure 4I.

An additional minor issue concerns the interpretation of the scd-2 experiments. The authors' findings do support a role for scd-2 signaling in the activation of daf-7 expression by un-ingestible food, but the data also suggest that scd-2 signaling is not essential for this effect, as there is still an effect in scd-2 mutants (Figure 4B).

Considering that most of previous Figure 4B is redundant with previous Figure 4D, we removed previous Figure 4B. Our current Figure 4 has redesignated previous Figure 4D as 4B. We have also added qualification to the text to indicate that other pathways may modulate the daf-7 expression response to ingested food in parallel to SCD-2 signaling.

Reviewer #2 (Public Review):

Summary:

In this work, Boor and colleagues explored the role of microbial food cues in the regulation of neuroendocrine-controlled foraging behavior. Consistent with previous reports, the authors find that C. elegans foraging behavior is regulated by the neuroendocrine TGF β ligand encoded by daf-7. In addition to its known role in the neuroendocrine/sensory ASI neurons, Boot and colleagues show that daf-7 expression is dynamically regulated in the ASJ sensory neurons by microbial food cues - and that this regulation is important for exploration/exploitation balance during foraging. They identify at least two independent pathways by which microbial cues regulate daf-7 expression in ASJ: a likely gustatory pathway that promotes daf-7 expression and an opposing interoceptive pathway, also likely chemosensory in nature but which requires microbial ingestion to inhibit daf-7 expression. Two neuroendocrine pathways known to regulate foraging (serotonin and PDF-1) appear to act at least in part via daf-7 induction. They further identify a novel role for the C. elegans ALK orthologue encoded by scd-2, which acts in interneurons to regulate daf-7 expression and foraging behavior. These

results together imply that distinct cues from microbial food are used to regulate the balance between exploration and exploitation via conserved signaling pathways.

Strengths:

The findings that gustatory and interoceptive inputs into foraging behavior are separable and opposing are novel and interesting, which they have shown clearly in Figure 1. It is also clear from their results that removal of the interoceptive cue (via transfer to non-digestible food) results in rapid induction of daf-7::gfp in ASJ, and that ASJ plays an important role in the regulation of foraging behavior.

We thank Reviewer 2 for underscoring the modulation of neuroendocrine gene expression in the ASJ neuron pair by distinct gustatory and interoceptive inputs derived from bacterial food that we show in Figure 1.

The role of the hen-1/scd-2 pathway in mediating the effects of ingested food is also compelling and well-interpreted. The use of precise gain-of-function alleles further supports their conclusions. This implies that important elements of this food-sensing pathway may be conserved in mammals.

We thank Reviewer 2 for emphasizing the implications of our study on SCD-2/ALK as well as the generation and use of gain-of-function scd-2 alleles based on oncogenic mutations in ALK.

Weaknesses:

What is less clear to me from the work at this stage is how the gustatory input fits into this picture and to what extent can it be strongly concluded that the daf-7 regulating pathways that they have identified (del-3/7, 5-HT, PDFR-1, scd-2) act via the interoceptive pathway as opposed to the gustatory pathway.

It follows from the work of the Flavell lab that del-3/7 likely acts via the interoceptive pathway in this context as well but this isn't shown directly - e.g. comparing the effects of aztreonam-treated bacteria and complete food removal to controls. The roles of 5-HT and PDFR-1 are even a bit less clear. Are the authors proposing that these are entirely parallel pathways? This could be explained in better detail.

We have added additional data regarding daf-7 expression from the ASJ neurons in the complete absence of food in the different mutant backgrounds noted by Reviewer 2. Data regarding daf-7 expression in the ASJ neurons under three distinct conditions—ingestible bacterial food, non-ingestible bacterial food, and the complete absence of food—enable the pairwise comparison of mutant data that allows for inference regarding the relative contributions of the genes to the interoceptive vs. gustatory pathways. In particular, effects on the interoceptive pathway can be inferred from the comparison of daf-7 expression on ingestible vs. non-ingestible food, whereas effects on the gustatory pathway can be inferred from the comparison of daf-7 expression on non-ingestible food vs. the absence of food (newly added).

These additional data are most informative for del-3; del-7 (Figure 1H), where the added data corroborate a role for these genes in the interoceptive pathway, consistent with the findings of the Flavell lab. Specifically, the observation that daf-7 expression levels are equivalent between wild-type and del-3;del-7 animals when there is no ingestible food (either no food or non-ingestible food conditions) suggest that DEL-3 and DEL-7 are functioning specifically to sense ingested food.

For pdf-1, the analysis of the gain-of-function allele suggest that this pathway may have a greater relative effect on the gustatory pathway compared with the interoceptive pathway

(Figure 3D). The robust upregulation seen in the *pdf-1(syb3826)* animals between animals on ingestible and non-ingestible food, suggests that the interoceptive regulation is functional in these mutants, while the lack of upregulation between no-food and noningestible-food conditions suggests that the gustatory pathway is affected.

The observations with the 5-HT biosynthesis mutant are most consistent with serotonin signaling affecting *daf-7* expression in the ASJ neurons through a mechanism that is parallel to the gustatory and interoceptive inputs into *daf-7* expression in the ASJ neurons, as *tph1(n4622)* animals appear to have an elevated baseline expression of *daf-7* in the ASJ neurons while retaining sensitivity to both gustatory and interoceptive food cues (Figure 3B).

The data with *scd-2* are consistent with a role in the epistatic interoceptive pathway, considering the roughly equivalent levels of *daf-7* expression in the ASJ neurons under all food conditions in *scd-2(syb2455)* animals (Figure 4B). However it is difficult to exclude the possibility that SCD-2 functions in both pathways or parallel to the gustatory and interoceptive inputs.

While we agree that our genetic analysis alone cannot distinguish between genes acting in parallel or directly in serial with the gustatory or interoceptive inputs, our data do establish that signaling through SCD-2, 5-HT or PDFR-1-dependent pathways can act on the same gene expression and signaling node (i.e. *daf-7* expression in the ASJ neurons) to modulate the effects of bacterial food inputs on foraging behavior, with the effects on *daf-7* expression in the ASJ neurons in *scd-2*, *tph-1* and *pdf-1* mutants correlating with their effects on roaming and dwelling behaviors.

It would also be helpful to elaborate more on why the identified transcriptional positive feedback loop is predicted to extend roaming state duration - as opposed to some other mechanism of increasing roaming such as increased probability of roaming state initiation. This doesn't seem self-evident to me.

Given that animals can exist in only two states, the increased probability of roaming state initiation would present as shorter dwelling states, which we do not see for *daf-7* mutants. As described in Flavell, et al., 2013, a decreased fraction of time roaming can be attributed to longer dwelling states, shorter roaming states, or both. Our positive feedback loop is predicted to extend roaming states because of the predicted effect of DAF-7 on stabilizing the roaming state.

*Related to this point is the somewhat confusing conclusion that the effects of *tph-1* and *pdf-1* mutations on *daf-7* expression are due to changes in ingestion during roaming/dwelling. From my understanding (e.g. Cermak et al., 2020), pharyngeal pumping rate does not reliably decrease during roaming - so is it clear that there are in fact lower rates of ingestion during roaming in their experiments?*

This is an interesting point. Despite consistent pumping rates, we still believe that roaming animals ingest less food than dwelling animals. For instance, dwelling animals are localized to areas with bacterial food, while roaming animals might traverse patches with no food where pumping does not result in food ingestion.

*If so, why does increased roaming (via *tph-1* mutation) result in further increases in *daf-7* expression in animals fed *aztreonam*-treated food (Fig 3B)?*

This is possibly because although roaming animals are eating less, when animals are on non-ingestible food, they're not eating at all, resulting in further *daf-7* upregulation.

Alternatively, there could be a direct signaling connection between the 5-HT/PDFR-1 pathways and daf-7 expression which could be acknowledged or explained.

Yes, this is certainly possible. We do not propose that all of the difference in daf-7 expression is due to changes in foraging behavior, but rather we are highlighting further instances of the correlation between daf-7 expression in the ASJ neurons and roaming. For instance, in the case of our tph-1 mutants, we see a relatively modest effect on daf-7 expression in the ASJ neurons but a large difference in the fraction of time roaming. This suggests that the magnitude of change in one (daf-7 expression in ASJ or roaming) does not predict the magnitude of the change in the other, but rather that they trend in the same direction.

Reviewer #3 (Public Review):

Summary:

In this interesting study, the authors examine the function of a C. elegans neuroendocrine TGF-beta ligand DAF-7 in regulating foraging movement in response to signals of food and ingestion. Building on their previous findings that demonstrate the critical role of daf-7 in a sensory neuron ASJ in behavioral response to pathogenic P. aeruginosa PA14 bacteria and different foraging behavior between hermaphrodite and male worms, the authors show, here, that ingestion of E. coli OP50, a common food for the worms, suppresses ASJ expression of daf-7 and secreted water-soluble cues of OP50 increases it. They further showed that the level of daf-7 expression in ASJ is positively associated with a higher level of roaming/exploration movement. Furthermore, the authors identify that a C. elegans ortholog of Anaplastic Lymphoma Kinase, scd-2, functions in an interneuron AIA to regulate ASJ expression of daf-7 in response to food ingestion and related cues. These findings place the DAF-7 TGF-beta ligand in the intersection of environmental food conditions, food intake, and foodsearching behavior to provide insights into how orchestrated neural functions and behaviors are generated under various internal and external conditions.

Strengths:

The study addresses an important question that appeals to a wide readership. The findings are demonstrated by generally strong results from carefully designed experiments.

We thank Reviewer 3 for the comments and interest in the work.

Weaknesses:

However, a few questions remain to provide a complete picture of the regulatory pathways and some analyses need to be strengthened. Specifically,

- 1. The authors show that diffusible cues of bacteria OP50 increase daf-7 expression in ASJ which is suppressed by ingestible food. Their results on del-3 and del-7 suggest that NSM neuron suppresses daf-7 ASJ expression. What sensory neurons respond to bacterial diffusible cues to increase daf-7 expression of ASJ? Since ASJ is able to respond to some bacterial metabolites, does it directly regulate daf-7 expression in response to diffusible cues of OP50 or does it depend on neurotransmission for the regulation? Some level of exploration in this question would provide more insights into the regulatory network of daf-7.*

The focus of our study has been on the modulation of *daf-7* expression in the ASJ neurons by distinct bacterial food cues and the downstream neuroendocrine circuitry that is influenced. The question of whether bacterial cues are directly sensed by the ASJ neurons remains unresolved by our study. However, we have previously demonstrated that the *daf-7* expression in the ASJ neurons induced by *P. aeruginosa* metabolites is likely the result of direct detection by the ASJ neurons. We would also note (and have added to the manuscript) the observation of Zaslaver et al. (2015), in which increased calcium transients were observed in the ASJ neurons in response to the withdrawal of *E. coli* OP50 supernatant, which is consistent with our observations of the effect of a soluble bacterial food signal on *daf-7* expression in the ASJ neurons.

1. *The results including those in Figure 2 strongly support that daf-7 in ASJ is required for roaming. Meanwhile, authors also observe increased daf-7 expression in ASJ under several conditions, such as non-ingestible food. Does non-ingestible food induce more roaming?*

Yes, this has been published by Ben Arous, et al., 2009. Figure 3C shows increased roaming on aztreonam-treated food. We have added specific mention of this in the text.

It would complete the regulatory loop by testing whether a higher (than wild type) level of daf-7 in ASJ could further increase roaming. The results in pdf-1 and scd-2 gain-of-function alleles support more ASJ leads to more roaming, but the effect of these gain-of-function alleles may not be ASJ-specific and it would be interesting to know whether ASJ-specific increase of daf-7 leads to a higher level of roaming. In my opinion, either outcome would be informative and strengthen our understanding of the critical function of daf-7 in ASJ demonstrated here.

We looked at roaming in animals with a *ptrx-1::daf-7* cDNA transgene in a wild-type background and did not see changes in the fraction of time animals roam. However, multiple experimental factors could contribute to our inability to detect an effect, including relative promoter strength and context of other variables that alter *daf-7* expression. Nevertheless, our data confirmed that ASJ neuron-specific expression of *daf-7* cDNA can increase roaming in a *daf-7* mutant background (Figure 2B).

We have also included an experiment (Figure 4I) looking at roaming in the *scd-2(syb2455)* gain-of-function animals in animals with *daf-7* deleted from the ASJ neurons. These results suggest that part of the increased roaming seen in these *scd-2(syb2455)* animals is specifically due to increased *daf-7* expression in the ASJ neurons.

1. *The analyses in Figure 4 cannot fully support "We further observed that the magnitude of upregulation of daf-7 expression in the ASJ neurons when animals were moved from ingestible food to non-ingestible food was reduced in scd-2(syb2455) to levels only about one-fourth of those seen in wild-type animals (Figure 4D)...", because the authors tested and found the difference in daf-7 expression between ingestible and non-ingestible food conditions in both wild type and the mutant worms. The authors did not analyze whether the induction was different between wild type and mutant. Under the ingestible food condition, ASJ expression of daf-7 already looks different in scd-2(syb2455).*

We appreciate the reviewer pointing out our lack of clarity in discussing our analysis of the data. The 4x difference represents the difference in fold change from ingested to noningested food in wild type and *scd-2(syb2455)* backgrounds. For wild-type animals, *daf-7* expression in the ASJ neurons on non-ingestible food is 8.1-times higher on non-ingestible food than on

ingestible food. In *scd-2(syb2455)* animals, this difference is 1.7 times. We have clarified this in the text.

1. *The authors used unpaired two-tailed t-tests for all the statistical analyses, including when there are multiple groups of data and more than one treatment. In their previous study Meisel et al 2014, the authors used one-way ANOVA, followed by Dunnett's or Tukey's multiple comparison test when they analyzed daf-7 expression or lawn leaving in different mutants or under different bacterial conditions. It is not clear why a two-tailed t-test was used in similar analyses in this study*

We have performed one-way ANOVAs for all comparisons included, and the results were largely consistent with what we found for t-tests. Ultimately, for our analysis we were most interested in pairwise comparisons and decided that t-tests would be most appropriate.

*Reviewer #1 (Recommendations For The Authors):

Line 170: For clarity, I suggest editing this to: "When animals are removed from edible food but are still exposed to soluble food signals, upregulation of daf-7..."

We have edited this in the text and appreciate the suggestion.

The authors report that pdf-1(syb3826) was retrieved from "a screen done in parallel to this work." syb3826 is a Suny Biotech allele, suggesting that this screen may not have been done in the authors' lab but rather outsourced. Some additional details might be useful.

This S325F allele was originally recovered as qd385 in an EMS screen performed in our lab. syb3826 is an independently generated Suny Biotech allele we ordered to confirm that the S325F substitution in PDFR-1 was responsible for our phenotypes. This has been clarified in the text.

Line 210: Please provide a citation for the screen that identified hen-1(qd259).

This is the first time the allele is being published. The screen is included in two theses from our lab, Meisel 2016 and Park 2019.

Line 214: It would be useful here to also mention the previously identified role of scd2 in sensory integration.

Yes, we have added this to the text. Additionally, we have included a couple of sentences in the discussion about how previous studies that have found a role for SCD-2 in sensory integration may instead be detecting the role for SCD-2 in food sensing, as many of the assays used for sensory integration are also sensitive to nutritional status of the animals.

*Line 271: Please provide a citation for the sex differences in food-leaving behavior (Lipton 2004 PMID 15329389 is the first careful characterization of this).
We have added this to the text.*