Neuronal and molecular substrates for optimal foraging in *Caenorhabditis elegans*

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Variation in food quality and abundance requires animals to decide whether to stay on a poor food patch or leave in search of better food. An important question in behavioral ecology asks when is it optimal for an animal to leave a food patch it is depleting. Although optimal foraging is central to evolutionary success, the neural and molecular mechanisms underlying it are poorly understood. Here we investigate the neuronal basis for adaptive food-leaving behavior in response to resource depletion in *Caenorhabditis elegans*, and identify several of the signaling pathways involved. The ASE neurons, previously implicated in salt chemoattraction, promote food-leaving behavior via a cGMP pathway as food becomes limited. High ambient O_2 promotes food-leaving via the O_2-sensing neurons AQR, PQR, and URX. Ectopic activation of these neurons using channelrhodopsin is sufficient to induce high food-leaving behavior. In contrast, the neuropeptide receptor NPR-1, which regulates social behavior on food, acts in the ASE neurons, the nociceptive ASH neurons, and in the RMG interneuron to repress food-leaving. Finally, we show that neuroendocrine signaling by TGF-β/DAF-7 and neuronal insulin signaling are necessary for adaptive food-leaving behavior. We suggest that animals integrate information about their nutritional state with ambient oxygen and gustatory stimuli to formulate optimal foraging strategies.

food-leaving decision | Marginal Value Theorem | sensory neurons

For most animals food is distributed unpredictably in patches of heterogeneous quality and quantity. As a food patch is depleted an animal must decide when it is advantageous to seek better feeding opportunities elsewhere. Understanding this decision is an important aim in behavioral ecology. Charnov's Marginal Value Theorem (MVT) suggests an animal's chosen strategy will reflect the energetic rewards gained upon encountering a better quality patch elsewhere, weighed against the energy expended (in locomotion and cessation of feeding) in traveling between patches (1). The MVT yields the "giving up time," the point in time when an animal should leave. Although optimal foraging behavior has been studied in many organisms, the neural basis for these decisions is not understood in any animal.

Caenorhabditis elegans feeds on microorganisms that grow on rotting material (2) and is adapted to exploit transient resources rapidly. Its simple nervous system and powerful genetics provide opportunities to define mechanisms underlying food patch-leaving choice at both molecular and circuitry levels. Several aspects of *C. elegans* foraging have been studied previously. Shtonda and Avery showed that *C. elegans* exercises food choice, selecting high quality food and leaving "harder-to-eat" bacteria (3). Wild strains of *C. elegans* can sense where food is thickest and aggregate there ("social" behavior) (4). These behaviors are attenuated in the N2 laboratory reference strain because of a gain-of-function mutation in the neuropeptide receptor *npr-1* gene that arose during laboratory domestication (5, 6). The gene *npr-1* also affects dispersal behavior in the presence of abundant food. Social worms disperse between food patches more readily than solitary worms (7). Recently, a polymorphism in *tyra-3*, encoding a G protein-coupled receptor for tyramine and octopamine, was shown to modify exploratory leaving behavior (8). Interestingly, wild isolates of *C. elegans* increase their dispersal as food concentration decreases (9), suggesting that these animals continuously assess the value of the patch. The value an animal places on a given patch is determined not only by its intrinsic nutritional value, but also by the costs involved in acquiring the food. Such costs may include exposure to environmental hazards and predation. How sensory inputs associated with these aversive cues modify food-leaving behavior is not understood mechanistically in any organism. Although our understanding of *C. elegans* ecology is rudimentary (10), recent work suggests that two environmental cues that modify foraging are ambient levels of O_2 and CO_2 (11–14). *C. elegans* avoids ambient O_2 concentrations close to 21%, a response that may ensure it avoids the surface with its associated hazards. Avoidance of high ambient O_2 is promoted by a cGMP signaling pathway in the AQR, PQR, and URX O_2-sensing neurons (11, 12, 15, 16). *C. elegans* also escapes environments with elevated CO_2 (13, 14). Several neurons have been implicated in this behavior, including the ciliated head neurons BAG, AFD, and ASE (14, 17, 18). CO_2 responses in these three neurons also involve cGMP signaling.

Here we establish a paradigm to study *C. elegans* food-leaving behavior specifically in response to resource depletion, in line with the MVT. Using targeted transgenic rescue of well-characterized mutants, we identify signaling pathways and sensory neurons regulating this behavior.

Results

Food-Leaving Is an Adaptive Exploratory Behavior Induced as Food Becomes Limiting. To investigate behavioral responses to resource depletion in *C. elegans*, we developed an assay to study food-leaving behavior (*Methods*). We used a dense bacterial lawn that, over 20 h, was gradually depleted by feeding animals. We filmed young adults to follow their behavior as food diminished (Fig. 1). Initially animals accumulated at the border, where food was thickest, and reversed to remain on food if the head, the tip of which is rich in chemoreceptors and mechanoreceptors, emerged from the food lawn. Over time, wild-type animals exhibited higher food-leaving as the food became scarcer (Fig. 1 *A* and *B*). This pattern of food-leaving is predicted by the adaptive benefits of leaving a diminishing food resource in search of a better quality patch.

We next sought to deconstruct food-leaving into more elemental behavioral components. Using videotracking software we quantified how the speed of animals, their reversal frequency all over the food patch and specifically at the border, their rate of arrival at the food border, the time they spend at the border, and the proportion of animals that leave food per encounter with the border, changed as they depleted food. Animals increased their speed from 25 to 35 μm/s as food decreased (Fig. 1*C*). This

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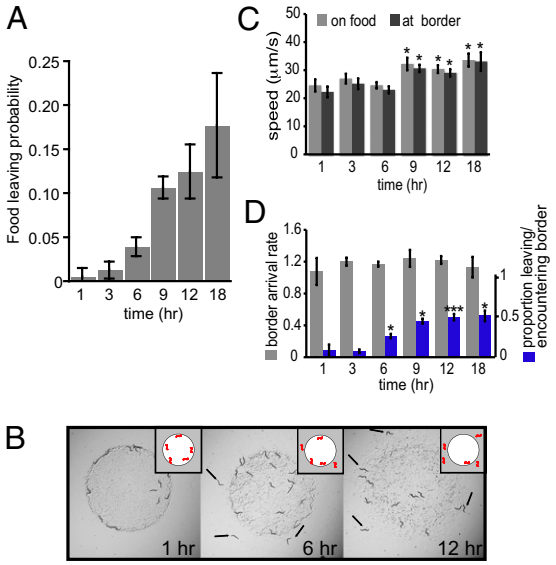

Fig. 1. Adaptive food-leaving in wild-type animals. (*A*) Food-leaving probability increases over time as animals deplete their food source. Food-leaving probability is calculated as the number of worms leaving per minute divided by the total number of worms on the food at start of that minute, averaged over 15 min (3). Error bars show SD. (*B*) Single frames from 1-, 6-, and 12-h movies and schematic drawings. The arrows point at animals outside of food or at the outer edge of the food border. (*C*) Speed on food and at the border, and the proportion of animals encountering the border that leave food increased over time (*D*). Error bars denote SEM (*C* and *D*). ***$P < 0.005$, **$P < 0.01$, *$P < 0.05$. $n = 6$ or more per strain.

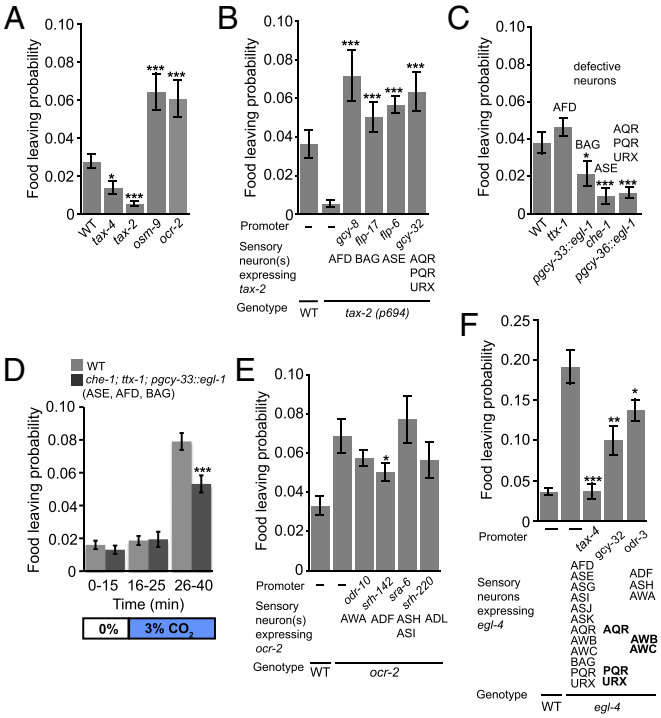

Fig. 2. Adaptive food-leaving is promoted by ASE, BAG, and the body cavity neurons AQR, PQR, and URX and suppressed by ADF neurons. (*A*) The *tax-4* and *tax-2* mutants show lower food-leaving than wild-type animals, whereas *osm-9* and *ocr-2* mutants show higher food-leaving. (*B*) Increased food-leaving of *tax-2(p694)* animals can be rescued by expressing *tax-2* cDNA in AFD (*pgcy-8*), BAG (*pflp-17*), ASE (an ASE-specific *pflp-6* fragment), and AQR, PQR, and URX (*pgcy-32*). (*C*) The *ttx-1* mutants behave similarly to wild-type animals with respect to food-leaving. In contrast, animals in which BAG neurons are ablated using a *pgcy-33::egl-1* transgene, as well as *che-1* mutants defective for ASE function, and animals genetically ablated for AQR, PQR, and URX neurons using *egl-1* expression from the *gcy-36* promoter stayed strongly on food. (*D*) After 15 min of airflow at 21% O_2, wild-type animals and *che-1;ttx-1 pgcy-33::egl-1* animals (which are defective in the CO_2 sensing neurons ASE, AFD, and BAG) were subjected to a shift to 3% CO_2 (21% O_2). This shift elicited a substantial increase in food-leaving after about 10 min that was partially suppressed in *che-1;ttx-1 pgcy-33::egl-1* animals. (*E*) High food-leaving in *ocr-2* mutants can be partially rescued by expressing *ocr-2* cDNA in ADF neurons. (*F*) The *egl-4* loss-of-function mutants show higher food-leaving than wild-type. Wild-type levels of food-leaving can be restored by expressing *egl-4* from the *tax-4* promoter. Expressing *egl-4* from the *gcy-32* promoter, which drives expression specifically in AQR, PQR, and URX neurons, or the *odr-3* promoter, which drives expression in AWA, AWB, AWC, ASH, and ADF neurons, results in partial rescue. Error bars denote SEM. ***$P < 0.005$, **$P < 0.01$, *$P < 0.05$. $n = 6$ or more per genotype.

increase in speed did not increase the rate at which animals encountered the food border (Fig. 1*D*); however, the proportion of animals that left food per border encounter progressively increased from 0.1 to 0.5 over time (Fig. 1*D*) as reversals at the border decreased (Fig. S1*A*).

These time-course data focused our subsequent studies on the behavior of animals that have been foraging on our assay plates for 6 h, because this represented an intermediate frequency of food-leaving that allowed us to measure both enhancement and suppression of food-leaving behavior.

Gustatory Neuron ASE, the O_2-Sensing Neurons AQR, PQR, and URX, and the CO_2-Sensing Neuron BAG Promote Adaptive Food-Leaving. Evaluation of a food source is likely to reflect integration of two kinds of sensory cues: external cues, associated with the food itself and its environmental context, and internal cues, such as neuroendocrine signals that communicate the animal's nutritional status. To initiate our circuitry dissection we sought first to disrupt sensory input from external cues.

Chemosensory transduction in *C. elegans* is generally mediated either by transient receptor potential V-like ion channels encoded by *osm-9* and its associated subunits encoded by the *ocr* genes (19, 20), or by cGMP-gated channels that incorporate subunits encoded by the *tax-4* and *tax-2* genes (21, 22). We asked whether mutations in these genes altered food-leaving behavior.

Loss-of-function mutations in either *tax-4* or *tax-2* reduced food-leaving (Fig. 2*A*). The *tax-2* mutants moved at speeds similar to wild-type and encountered the border at a similar rate in our assay (Fig. S1*B*); however, the proportion of animals at the border that left the food per edge encounter was reduced compared with wild-type (Fig. S1*C*). In contrast, mutations in *osm-9* or *ocr-2* increased food-leaving (Fig. 2*A*). In *osm-9* mutants the proportion of animals leaving food per edge encounter was not altered compared with wild-type controls, but animals showed elevated speed and increased border arrival rate, accounting for their higher food-leaving rate (Fig. S1*C*).

Both *tax-4* and *tax-2* are coexpressed in about 12 sensory neurons (21, 22). The *tax-2* allele we used, *tax-2(p694)*, is a promoter deletion that disrupts the function of only a subset of these sensory neurons, namely AQR, PQR, URX, ASE, AFD, and BAG (17, 21, 23). This finding suggested that one or more of these neurons promoted food-leaving behavior. To identify these neurons, we sought to rescue the food-leaving defect in *tax-2 (p694)* mutants by expressing *tax-2* cDNA using cell-specific promoters. Expressing *tax-2* in AFD alone, BAG alone, ASE alone, or in AQR, PQR, and URX restored food-leaving to *tax-2* mutants (Fig. 2*B* and Fig. S1*E*). These data suggested that each of these neurons can promote food-leaving behavior.

To extend our studies, we examined the consequences of functionally ablating each neuron identified above (Fig. 2*C*). We genetically disrupted AFD neurons using a mutation in *ttx-1*; *ttx-1* encodes a homeodomain transcription factor of the *otd*/*otx* subclass that is specifically required to specify AFD neurons (24).

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The *ttx-1* mutants exhibited wild-type food-leaving behavior. Animals in which the BAG neurons were ablated by cell-specific expression of the *egl-1* cell-death gene exhibited significantly less food-leaving than wild-type animals. Strikingly, animals defective in *che-1*, a zinc-finger transcription factor required to specify the ASE neurons (25), or lacking AQR, PQR, and URX because of specific expression of *egl-1* in these O_2-sensing neurons, resulted in animals that stayed strongly on food (Fig. 2*C*). Thus, our cell-disruption experiments, like our *tax-2* rescue data, suggest that BAG, ASE, and one or more of the AQR, PQR, and URX neurons inhibit food-leaving. Interestingly, whereas the rescue experiments suggested that any one of the BAG, ASE, or AQR, PQR, or URX neurons was sufficient to restore food-leaving behavior to *tax-2(p694)* mutants, our cell-ablation/mis-specification experiments suggested that each of these neurons or neuron groups was necessary for wild-type food-leaving rates. One explanation for this difference was that the *tax-2(p694)* mutation disrupted a neural input that suppresses food-leaving probability. The likeliest candidate for this role was AFD. Consistent with this finding, disrupting the function of AFD using a *ttx-1* mutation suppressed the food-leaving phenotypes associated with loss of BAG or ASE (Fig. S2 *A* and *B*). These data suggest that AFD can act antagonistically to BAG and ASE to inhibit food-leaving behavior. Although these results contrast with our earlier *tax-2* rescue data implicating AFD in promoting food-leaving, recent work has shown that the AFD neurons can elicit opposite behavioral outcomes, depending on the level of activation (26).

Our data on the role of ASE, BAG, and AFD in food-leaving were highly reminiscent of a recent study implicating these neurons in CO_2 avoidance (13). All three neurons are CO_2 sensors. The neurons are activated by elevated CO_2 via a cGMP signaling pathway and promote avoidance of CO_2, although under some circumstances AFD can also inhibit avoidance of high CO_2. These parallels prompted us to examine if high ambient CO_2 levels altered food-leaving probability. Switching CO_2 concentration from 0 to 3% induced a high rate of food-leaving (Fig. 2*D* and Fig. S2*C*). This increased food-leaving in response to CO_2 was reduced in ASE-, AFD-, and BAG-defective animals. Thus, one way that BAG, ASE, and AFD neurons may regulate food-leaving is by responding to elevated CO_2 levels associated with microbial food or worm respiration.

We next investigated sensory neurons that prevent animals from leaving a food patch prematurely. The *osm-9* and *ocr-2* mutants left food more than wild-type. Both *ocr-2* and *osm-9* are coexpressed in six pairs of sensory neurons: ADF, ADL, ASH, AWA, PHA, and PHB (19, 20). Expressing *ocr-2* cDNA from neuron-specific promoters in four of these neurons individually implicated ADF neurons in food-leaving behavior (Fig. 2*E*), although selective expression of *ocr-2* in ADF rescued the *ocr-2* phenotype only partially. These results suggest that *ocr-2* functions in ADF neurons to inhibit food-leaving, but that other *ocr-2*–expressing neurons are also required for the wild-type food-leaving pattern.

The cGMP-dependent protein kinase, encoded in *C. elegans* by the *egl-4* gene, is a conserved modulator of food-related behaviors (27). The *egl-4(lf)* mutants spend more time roaming on food than wild-type animals (28). EGL-4 is also required for odor adaptation in the olfactory neuron AWC (29). We found that *egl-4(lf)* mutants exhibited increased food-leaving (Fig. 2*F*). This phenotype was associated with elevated locomotory activity on food, higher encounter frequency with the bacterial lawn border, reduced border reversal rate, and increased probability of food-leaving per border encounter (Fig. S2 *D–H*). Expressing *egl-4* cDNA under the control of the *tax-4* promoter rescued all these phenotypes (Fig. S2 *D–H*). Expressing *egl-4* in only AQR, PQR, and URX partially rescued these phenotypes. Expressing *egl-4* under the control of the *odr-3* promoter, which drives expression in AWA, AWB, AWC, ASH, and ADF also partially rescued these phenotypes. In summary, our results suggest that cGMP signaling in the ASE, AQR, PQR, URX, and BAG neurons mediated by a cyclic nucleotide channel containing the TAX-2 subunit promotes food-leaving, and that *ocr-2* and *egl-4* prevent premature food-leaving in ADF and in *tax-4*–expressing neurons (in particular AQR, PQR, and URX), respectively.

Insulin and TGF-β, Mediators of Nutritional State and Environmental Conditions, Respectively, Modulate Food-Leaving. We hypothesized that the food-leaving decision would be regulated by post-ingestive inputs (e.g., nutritional state or feeding history). Insulin signaling has previously been linked to perception of feeding state and food-related behavioral plasticity (30–33). This finding prompted us to analyze adaptive food-leaving in mutants defective in the insulin pathway. Mutants in the *daf-2* insulin receptor (34) stayed on food more than wild-type (Fig. 3*A*). Mutants in the PI3K *age-1*, one of the known downstream targets of insulin signaling, behaved similarly (35), suggesting AGE-1 mediated the affect of the DAF-2 receptor, although we cannot exclude that AGE-1 is activated by other insulin independent pathways. In contrast, mutants in *daf-16*, encoding the Forkhead box O transcription factor inhibited by insulin-like signaling (36), exhibited higher food-leaving behavior (see Fig. S3 for behavioral parameters of *daf-2* and *daf-16* mutants). The *daf-2;daf-16* double-mutants had a phenotype closer to wild-type. The incomplete epistasis of *daf-16* over *daf-2* suggests that *daf-2* promotes food-leaving by mechanisms that are only partly dependent on *daf-16*. To determine the site of action of DAF-16,

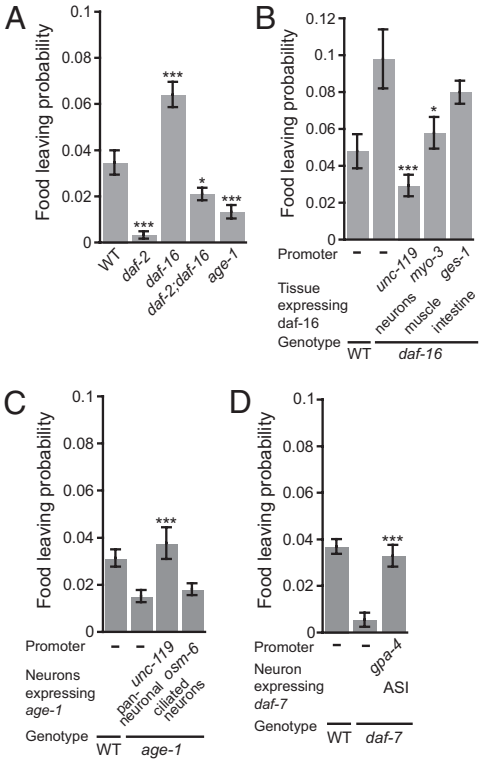

Fig. 3. Neuronal insulin and TGF-β signaling from the ASI neurons promote food-leaving. (*A*) The *daf-2* and *age-1* mutants exhibit reduced food-leaving compared with wild-type animals. The *daf-16* mutants exhibit higher food-leaving. *daf-2* suppresses the high food-leaving phenotype of *daf-16* mutants. (*B*) Expression of *daf-16* in all neurons from the *unc-119* promoter restores wild-type food-leaving. Expression of *daf-16* in muscle from the *myo-3* promoter shows partial rescue. Intestinal expression of *daf-16* from the *ges-1* promoter does not rescue the food-leaving phenotype of *daf-16* mutants. (*C*) *age-1* expression from the *unc-119* promoter, but not the *osm-6* promoter, restores wild-type food-leaving to *age-1* mutants. (*D*) The *daf-7* mutants exhibit reduced food-leaving. Wild-type food-leaving can be restored by expressing *daf-7* in the ASI neurons, using the *gpa-4* promoter. Error bars denote SEM. ***$P < 0.005$, *$P < 0.05$. $n = 6$ or more per strain.

we restored its expression in different tissues to *daf-16* mutant animals. Neuronal expression of *daf-16*, but not intestinal expression, conferred wild-type food-leaving to *daf-16* mutants (Fig. 3*B*). There was also partial rescue by expression of *daf-16* in body muscle in the *daf-16* mutants. Similarly, neuronal expression of an *age-1* transgene in *age-1* mutants restored wild-type food-leaving behavior (Fig. 3*C*). In contrast, expressing *age-1* in all ciliated neurons using the *osm-6* promoter (37), using a transgene previously shown to rescue other *osm-6* phenotypes, did not rescue the food-leaving defect. These data support a neuronal role for insulin signaling in food-leaving behavior, possibly in interneurons, although our negative results with the *posm-6::age-1* transgene does not allow us to rigorously exclude a role in sensory neurons.

Signaling via the TGF-β–like ligand DAF-7 transduces external conditions. Food shortage, high population density, and dauer pheromone inhibit *daf-7* expression and promote dauer formation (38, 39). As adults, *daf-7* mutants accumulate more fat and have a reduced pharyngeal pumping rate (40). Adaptive food-leaving was strongly reduced in *daf-7* mutants (Fig. 3*D*); *daf-7* is expressed in one pair of head sensory neurons, ASI (38, 39). Reintroducing *daf-7* expression in ASI in *daf-7* mutants completely restored wild-type food-leaving. Thus, TGF-β signaling from the ASI neurons promotes food-leaving behavior.

Neuropeptide Signaling via NPR-1 in ASH and RMG Control Food-Leaving Behavior. The neuropeptide receptor NPR-1 regulates food-related behaviors, such as aggregation (social feeding) (4), and has been implicated in *C. elegans* dispersal (7). The *npr-1* mutants had much higher food-leaving than wild-type (Fig. 4*A*). This phenotype was associated with much higher speed and border-arrival rates (Fig. 4*B*; see Fig. S4 for more behavioral parameters). The food-leaving defect in *npr-1* mutants, as well as the speed and border-arrival rate phenotypes, were suppressed by a mutation in *gcy-35*, which encodes an atypical soluble guanylate cyclase that mediates O_2 sensing in the body cavity neurons AQR, PQR, and URX (11, 41). Restoring *gcy-35* expression in the body cavity neurons reverted food-leaving behavior of *gcy-35;npr-1* to *npr-1* behavior, suggesting that the high food-leaving in *npr-1* mutants requires *gcy-35* activity in AQR, PQR, and URX (Fig. 4*A*).

FMRF-amide (Phe-Met-Arg-Phe-NH_2)-related peptides encoded by the *flp-18* and *flp-21* genes have been implicated as NPR-1 ligands (42, 43). However, a double-mutant *flp-18; flp-21* did not show higher food-leaving than wild-type, suggesting that neuropeptides other than FLP-18 and FLP-21 regulate food-leaving mediated by NPR-1.

To identify the cells where *npr-1* activity is required for adaptive food-leaving, we expressed *npr-1* using neuron-specific promoters in *npr-1* mutants. The *ncs-1* promoter drives expression in a subset of *npr-1*–expressing neurons: ASE, ASG, PHA, PHB, and RMG (44). Expression of *npr-1* from this promoter restored wild-type food-leaving behavior in *npr-1* mutants (Fig. 4*C*), as did expression of *npr-1* in the inter/motor neuron RMG and in the polymodal nociceptive neuron ASH. Expression of *npr-1* in either ASEL or ASER, from the *gcy-7* and *gcy-5* promoters,

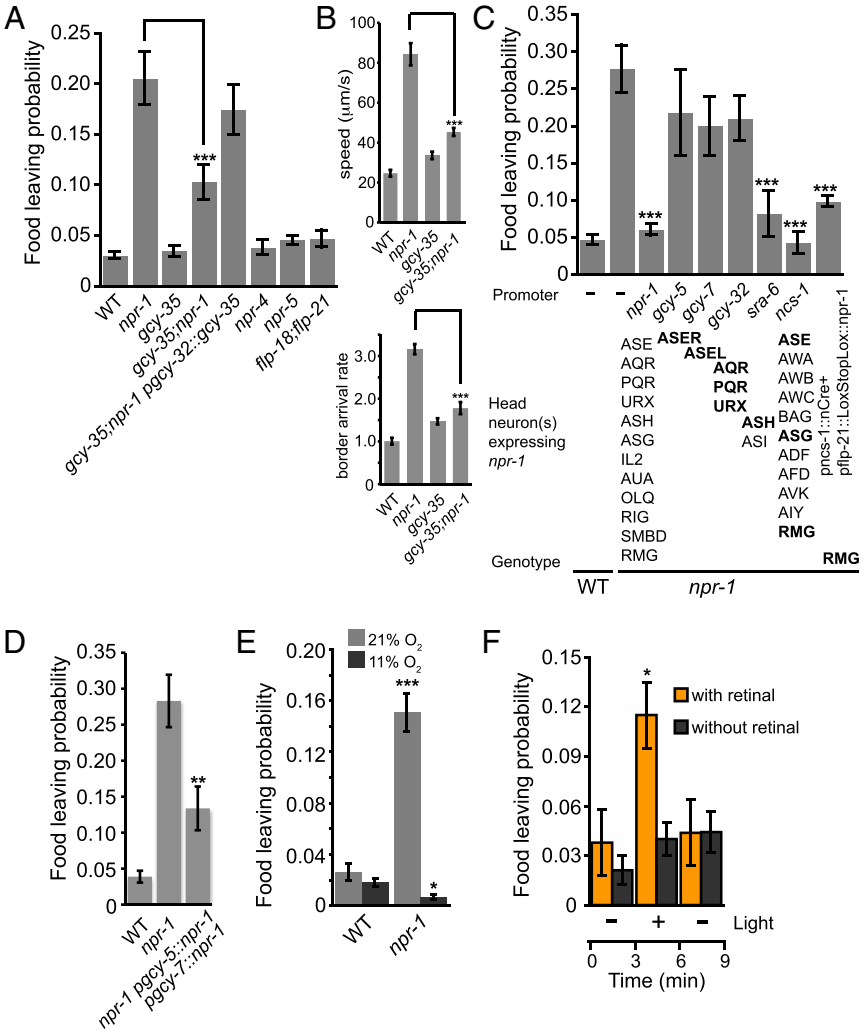

Fig. 4. The neuropeptide receptor NPR-1 functions in ASE, ASH, and RMG neurons to inhibit food-leaving and high ambient O_2 or ectopic activation of AQR, PQR, and URX induces high food-leaving. (*A*) The *npr-1* mutants show higher food-leaving than wild-type animals. *gcy-35* partially suppresses *npr-1* food-leaving. Expression of *gcy-35* from the *gcy-32* promoter in *gcy-35; npr-1* mutants shows that *gcy-35* acts in AQR, PQR, and URX to suppress the high food-leaving of *npr-1* mutants. Neither a *flp-18; flp-21* double-mutant nor mutants in their additional receptors *npr-4* and *npr-5* (50) showed higher food-leaving than wild-type. (*B*) The *npr-1* mutants show increased speed and border-arrival rate, which is suppressed in *gcy-35;npr-1* mutants. (*C*) Wild-type food-leaving can be restored in *npr-1* mutants by expressing *npr-1* specifically in ASH (and ASI) from the *sra-6* promoter, and in RMG using Cre-mediated recombination (51). Full rescue is also achieved from the *ncs-1* promoter, driving expression in RMG and 10 other neurons. (*D*) Expression of *npr-1* in both ASEL and ASER partially rescue food-leaving in *npr-1* mutants. (*E* and *F*) Food-leaving is context-dependent. (*E*) Wild-type and *npr-1* mutants were recorded at 21% oxygen and then switched to 11% oxygen in a Perspex chamber. Food-leaving of *npr-1* mutants was reduced at low oxygen tension, when the AQR, PQR, and URX neurons are less active (15, 16). (*F*) Ectopic stimulation of AQR, PQR, and URX neurons in *npr-1 lite-1* mutants by channelrhodopsin activation is sufficient to induce high food-leaving in animals kept at 11% oxygen. Animals were filmed 3 min before and after light activation. The orange bars denote the addition of the necessary cofactor, retinal. Error bars denote SEM. ***$P < 0.005$, **$P < 0.01$, *$P < 0.05$. $n = 6$ or more.

respectively, did not rescue *npr-1* mutants, but expression in both ASEL and ASER did (Fig. 4*D*). Expression of *npr-1* in the body cavity neurons did not significantly rescue the adaptive food-leaving behavior. This result implicates the ASE, ASH, and RMG neurons in the regulation of adaptive food-leaving behavior. We conclude that *npr-1* prevents food-leaving in ASE, ASH, and RMG neurons and that in the absence of *npr-1*, high food-leaving partly reflects the activity of the *gcy-35* O_2 sensor in the AQR, PQR, and URX neurons.

Activation of the Body Cavity Neurons Is Sufficient to Induce Food-Leaving. Ca^{2+} imaging studies have shown that AQR, PQR, and URX are activated by high ambient O_2 (15, 16). To investigate the role of the O_2-sensing body cavity neurons AQR, PQR, and URX in adaptive food-leaving, we first examined whether increased food-leaving by *npr-1* mutants was dependent on ambient O_2 levels. We found that *npr-1* mutants left food less when ambient O_2 was at 11%, consistent with AQR, PQR, and URX being less active at low O_2 (Fig. 4*E*). This result, together with our observation that mutations in the *gcy-35* O_2 sensor suppressed the food-leaving phenotype of *npr-1* animals, suggests that activation of the O_2 signaling pathway in AQR, PQR, and URX when animals are in 21% ambient O_2 promotes food-leaving.

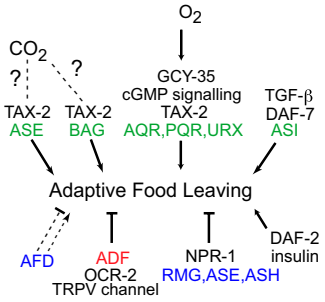

Fig. 5. Model for regulation of adaptive food-leaving behavior. The BAG, ASE, and AQR, PQR, URX neurons promote food-leaving via TAX-2/TAX-4. Food-leaving is also promoted by the ASI neurons via DAF-7 and by neuronal insulin signaling. High CO_2 and O_2 promote food-leaving. Food-leaving is suppressed by the ADF neuron via OCR-2, the AFD neuron and by NPR-1 neuropeptide signaling in ASE, ASH, and RMG neurons.

To directly test this hypothesis we expressed channelrhodopsin [ChR2] in the body cavity neurons of *npr-1* mutants and light-activated these neurons while animals were at 11% oxygen. Light stimulation induced a dramatic increase in food-leaving (Fig. 4*F*). This finding is consistent with the *tax-2* rescue experiments in AQR, PQR, and URX and suggests that activation of AQR, PQR, and URX is sufficient to induce high food-leaving behavior.

Discussion

A central question in behavioral ecology is what controls the timing of an animal's decision to leave a depleting resource patch. We find that *C. elegans* conforms to expectations, increasing its food-leaving probability as food becomes depleted, when a negative energy budget may increase the adaptive value of such risky behaviors (1, 45). This behavior, which we term "adaptive food-leaving," has the hallmarks of an optimal patch-leaving strategy. We identify a small set of neurons and signaling molecules that control adaptive food-leaving as food becomes limited (see Fig. 5 for our model).

The ASE gustatory neurons promote food-leaving. In well-fed animals the ASE neurons mediate chemoattraction to water-soluble cues. However, several studies have shown that food withdrawal can turn this attraction to salt into repulsion (33, 46–48). Our work suggests this gustatory plasticity occurs not only upon food withdrawal but also in feeding animals experiencing a gradual decrease in food patch quality. The work on gustatory plasticity has shown that the switch in preference involves signaling by the *daf-2* insulin receptor in ASER (33). Our data also implicate *daf-2* signaling in promoting adaptive food-leaving, although we have not identified an anatomical focus for this action. The BAG sensory neurons, which are exquisitely sensitive to rises in CO_2 (17, 18), also promote food-leaving. BAG neurons, as well as the ASE neurons, are tonically activated by elevated CO_2 (17). One possibility is that adaptive food-leaving in our assay partly reflects animals avoiding CO_2 generated by bacteria and other nematodes. Recent work has also implicated BAG as a modulator of food-leaving behavior, but suggests it inhibits food-leaving (8). However, these results were obtained in a different genetic background (in the presence of an *npr-1* allele derived from the Hawaiian wild strain) and under significantly less food-restricted conditions (8), so the data are not directly comparable.

The body cavity neurons AQR, PQR, and URX modulate adaptive food-leaving behavior, promoting food-leaving at high ambient O_2. These neurons are activated by high ambient [O_2] (15, 16) and promote reversals and turns when O_2 levels rise, as well as high locomotory activity (increased roaming) if the *npr-1* neuropeptide receptor is defective (12, 16). These neurons promote food-leaving in both *npr-1(N2)* and *npr-1(null)* mutant backgrounds. Moreover, we find that ectopic activation of AQR, PQR, and URX neurons using channelrhodopsin rapidly induces food-leaving. The effects of AQR, PQR, and URX on food-leaving depend on GCY-35, an atypical soluble guanylate cyclase that mediates O_2 responses (11) and is also required for the increased roaming of *npr-1* mutants on food (16). In *npr-1* animals, adaptive food-leaving can be directly modulated by altering ambient O_2: high O_2 is associated with rapid food-leaving, whereas low O_2 is associated with low food-leaving. Regulation of food-leaving by ambient O_2 may allow animals to balance their need to acquire food with avoiding a hazardous environment at the surface and accumulating in buried but food-containing environments.

The role of AFD neurons in food-leaving is more complex. AFD has been studied extensively for its role in temperature sensing, but recent data indicate it is also a CO_2 sensor that promotes CO_2 avoidance in some contexts and inhibits CO_2 avoidance in others (17). Our data suggest that similarly, AFD can both promote and suppress food-leaving depending on the context. More specifically, we find that AFD promotes food-leaving when ASE and BAG neurons are defective, but inhibits food-leaving if either ASE or BAG neurons are functional. A recent optogenetic study has shown that the level of activation of AFD determines whether it evokes attractive or repulsive behavior (26). Strong activation of AFD results in weaker activation of AIY, a major postsynaptic target of AFD, and avoidance behavior. In contrast, attenuating the activation of AFD using halorhodopsin leads to strong activation of AIY and attraction behavior. AIY interneurons receive synaptic input not only from AFD but also from the ASE and BAG neurons (49). We suggest that this common circuitry explains why the behavioral effects of AFD on food-leaving depend on the activity state of ASE and BAG. One caveat in our experiments is that we are using a mutation in *ttx-1* to genetically disrupt AFD, and we cannot be sure that this is equivalent to ablating the neuron.

In summary, we address the neuronal and molecular basis for food-leaving, a clearly defined and theoretically predictable aspect of animal behavior. Optimal forging has been widely studied at the behavioral level and modeled extensively; *C. elegans* provides an opportunity to test predictions made by these studies by manipulating its nervous system.

Methods

Strains. Nematodes were grown at 20 °C under standard conditions. All strains used can be found as *SI Methods*.

Behavioral Assays. Low peptone [5% of regular amount of bactopeptone (4)] nematode growth media plates were seeded 2 d before assay with 25 μL of OP-50 saturated overnight culture (from the *Caenorhabditis* Genetics Center) in 2XYT. Twenty young adults were transferred to the assay plates and allowed to feed for 6 h [except for the timescale (Fig. 1) and the channelrhodopsin experiment], after which they were recorded for 15 min or placed in a Perspex chamber for the O_2 and CO_2 experiments. The food-leaving probability was calculated as described by Shtonda and Avery (3). Briefly: the ratio between number of worms leaving per minute divided by the total number of worms on the food at start of that minute was calculated and averaged over 15 min. A minimum of six assays was done per genotype. All statistical analyses were conducted using Minitab15 software.

To test the validity of pooling data from different assay days, a Kruskal–Wallis test was conducted. Because food-leaving probability for each strain did not differ significantly between assay days ($P > 0.05$), statistical analyses were performed on pooled data sets used the *t* test.

Details can be found in *SI Methods*.

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