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RESEARCH ARTICLE

The olfactory neuron AWC promotes avoidance of normally palatable food following chronic dietary restriction

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ABSTRACT

Changes in metabolic state alter foraging behavior and food preference in animals. Here, I show that normally attractive food becomes repulsive to *Caenorhabditis elegans* if animals are chronically undernourished as a result of alimentary tract defects. This behavioral plasticity is achieved in two ways: increased food leaving and induction of aversive behavior towards food. A particularly strong food avoider is defective in the chitin synthase that makes the pharyngeal lining. Food avoidance induced by underfeeding is mediated by cGMP signaling in the olfactory neurons AWC and AWB, and the gustatory neurons ASJ and ASK. Food avoidance is enhanced by increased population density and is reduced if the animals are unable to correctly interpret their nutritional state as a result of defects in the AMP kinase or TOR/S6kinase pathways. The TGF-β/DBL-1 pathway suppresses food avoidance and the cellular basis for this is distinct from its role in aversive olfactory learning of harmful food. This study suggests that nutritional state feedback via nutrient sensors, population size and olfactory neurons guides food preference in *C. elegans*.

KEY WORDS: Aversion, Foraging, Nutritional state, Sensory neurons, TFG-β/DBL-1 pathway

INTRODUCTION

Animals need to integrate sensory information on food quality with post-ingestive feedback to allow them to select food that best supports growth and reproduction. If food is nutrient deficient or harmful, animals develop aversion to that particular food (Provenza, 1996). How this aversion develops mechanistically is unclear. In *Caenorhabditis elegans*, rejection of pathogenic food is a learnt behavior (Zhang et al., 2005a). It takes about 4 h for animals to learn to avoid pathogenic bacteria. Similarly, preference for good quality food over hard to eat food develops over time in *C. elegans* (Shtonda and Avery, 2006), suggesting that the animals make their decisions based on their internal metabolic state as well as external information. How the internal nutritional state is used to assign values to sensory information on food quality and abundance is poorly understood. Here, I show that feeding-defective mutants both leave and avoid good food. I use this food-avoidance paradigm to dissect the neuronal basis for this behavior at a sensory level. Food avoidance in feeding-defective animals is primarily mediated by cGMP signaling in the odor-sensing neurons AWC and AWB. A second set of neurons, ASJ and ASK, function together to promote food avoidance. Avoidance behavior is delayed if the animals are unable to monitor their nutritional state correctly, or if they are defective in *hen-1*, a secreted protein with an LDL motif previously implicated in sensory integration and learning. In addition, I demonstrate that the TGF-β/DBL-1 pathway, which promotes olfactory learning of harmful bacteria (Zhang and Zhang, 2012), suppresses food avoidance in well-fed animals by mechanism that is at least partly distinct from its role in olfactory learning.

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Received 12 November 2013; Accepted 17 February 2014

RESULTS

Feeding-defective mutants leave and avoid good food

How do animals adjust their behavior to long-term changes in nutritional state? Wild-type hermaphrodite *C. elegans* accumulate strongly on abundant food and do not leave until food starts to deplete (Milward et al., 2011). Chronic reduction of food intake and hence reduced nutritional state (or dietary restriction) found in feeding-defective mutants such as *eat-2*, which lack an acetylcholine receptor subunit specifically expressed in the pharyngeal muscle (McKay et al., 2004), and *bav-1* (see below) induced a strong food-leaving response even in plentiful food conditions (Fig. 1A,B). Other feeding-defective mutants such as *pha-2* (Avery, 1993; Mörck et al., 2004) and *eat-6* (Avery, 1993; Davis et al., 1995) behaved similarly, and left good food (Fig. 1C). These chronically food-deprived animals accumulate outside normally palatable food, suggesting that as well as leaving food, they actively avoid it (Fig. 1C,F, supplementary material Movie 1). The strain GE337 shows particularly strong accumulation outside food. I outcrossed this strain and found this phenotype was associated with an allele of a gene I called *bav-1*, for *b*acterial *av*oidance-*1*. Like feeding-defective mutants, *bav-1* animals accumulated in a ring just outside food at higher population densities (Fig. 1F). Importantly, these chronically underfed animals such as *eat-2* and *bav-1* could still respond to immediate hunger: 1 h of food deprivation drastically reduced their feeding-defective behavior (Fig. 1A). Thus, chronically underfed animals can still respond to the presence or absence of food. However, chronic undernourishment appears to change *C. elegans*' response to the food from attraction to avoidance.

To explore food-avoidance behavior more closely, I monitored how animals behaved as they approached the bacterial food lawn. Wild-type animals usually entered the food lawn, and only occasionally reversed and turned away from it. By contrast, chronically underfed animals often reversed and turned when approaching the bacterial lawn, or 'hesitated' close to the food edge (hesitation occurs when animals stall close to the food edge and lift their noses and swing their heads) (Fig. 1E). These data suggest that chronic malnourishment alters animals' perception of food quality, leading to food aversion.

To distinguish food-leaving behavior from food avoidance, we asked whether mutants exhibiting high food leaving also invariably avoided food. *osm-9* mutants show high food-leaving behavior, both

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The Journal of Experimental Biology (2014) doi:10.1242/jeb.099929

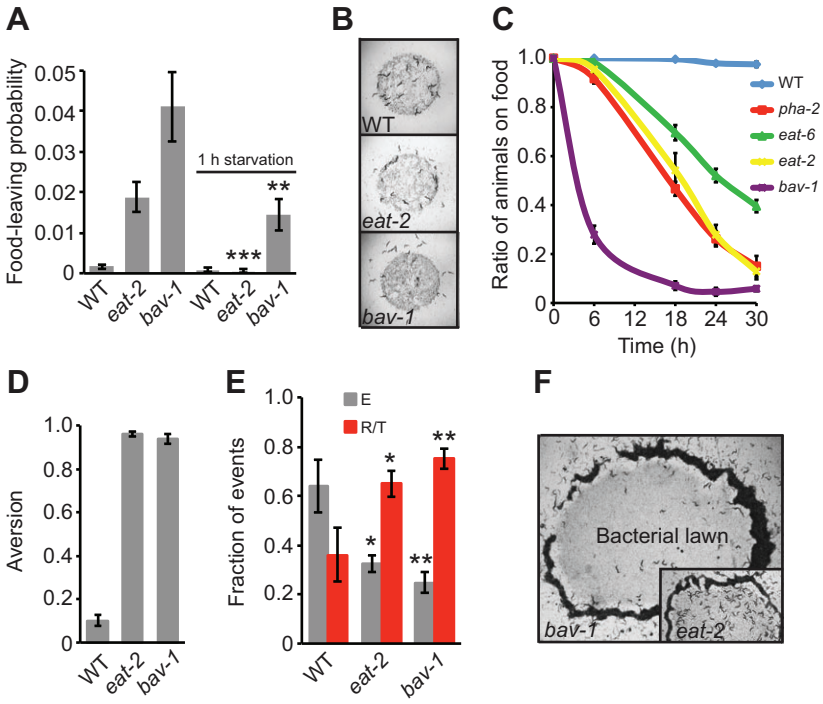

Fig. 1. Chronic dietary restriction induces *Caenorhabditis elegans* to leave and avoid palatable food. (A) *eat-2* and *bav-1* animals exhibit increased food leaving on abundant food. Withdrawing food for 1 h reduced subsequent food leaving in *eat-2* and *bav-1* animals. Each genotype was assayed at least six times. WT, wild-type. Error bars represent s.e.m. Student's *t*-test compares each genotype under the two different conditions: **P*<0.05, ***P*<0.01, ****P*<0.005. (B) Distribution of animals on and off food at 10 h. (C) Feeding-defective mutants (*pha-2*, *eat-6*, *eat-2* and *bav-1*) accumulate off food. (D) Aversion score of *eat-2* and *bav-1*. (E) Accumulation outside food is associated with increased avoidance behavior when attempting to re-enter the food lawn. Reversals and turns (R/T) were scored manually from recordings at 24 h. At this time point, food depletion leads to high rates of food leaving in WT animals. Number of attempts to re-enter food lawn scored: WT (N2), *N*=63; *eat-2*, *N*=138; *bav-1, N*=179. E, entry into food. (F) At high population density, *eat-2* and *bav-1* animals form an avoidance ring outside the food lawn.

on a depleting food source (Milward et al., 2011) and on a thick lawn (supplementary material Fig. S1A). Although 40% of *osm-9* animals were found off food after 24 h on a bacterial lawn, these animals did not show increased reversals and turns upon food approach (supplementary material Fig. S1B). By contrast, in chronically underfed animals there was an increase in both food leaving and aversive behavior to food. *eat-2* and *bav-1* have an aversion score [N^{off}/N^{total}, as described elsewhere (Melo and Ruvkun, 2012)] (Fig. 1D) of close to 1, meaning that at the end of the assay almost 100% of the animals are off food.

The food avoider *bav-1* is a mutant in the pharynx-specific chitin synthase, *chs-2*

In order to understand why *bav-1* animals left and avoided food strongly, I characterized their behavior in more detail. One simple explanation for the avoidance phenotype could be that *bav-1* mutants failed to respond the food-associated cues. However, amphid neurons of *bav-1* mutants appear to be normal as judged by dye-filling (supplementary material Fig. S2A–C), and the mutants responded normally to a wide range of sensory stimuli (Fig. 2A–F). Thus, *bav-1* animals can sense and respond effectively to individual chemosensory cues. However, *chs-2* mutants appeared pale, suggesting that they have less fat deposited, and behaved like starved animals (e.g. exhibiting increased nose lifting). Nile Red staining of fixed animals (Fig. 2G,H) as well as Sudan Black staining (data not shown) confirmed that *bav-1* mutants have very low fat deposits, much like feeding-defective animals.

To gain more insight into the *bav-1* phenotype, I mapped the mutation and identified the *bav-1* locus by SNP-mapping and Illumina sequencing. The *bav-1* (*mbd1*) allele was associated with a

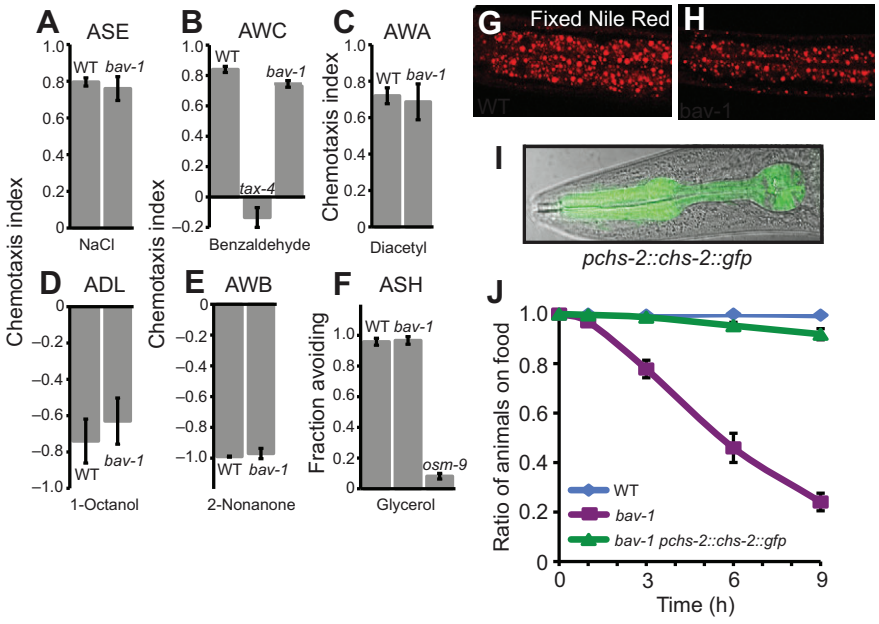

Fig. 2. *bav-1* disrupts chitin synthase-2, *chs-2*. (A–F) *bav-1* animals exhibit wild-type-like responses to different chemical cues: NaCl, benzaldehyde, diacetyl, 1-octanol, 2-nonanone and glycerol. All assays were carried out in the absence of food. (G,H) Fat staining of wild-type and *bav-1* mutants. Fixed Nile Red staining of L4 wild-type (G) and *bav-1* (H) larvae. Images show the intestine posterior to the grinder. (I) *chs-2* is expressed specifically in the pharynx. (J) The *bav-1* food-avoidance phenotype can be rescued by expressing genomic *chs-2* under its own promoter. Error bars represent s.e.m.

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G→A 3′ splice site mutation at position 3846 of the gene for chitin synthase 2, *chs-2*. *chs-2* is one of two chitin synthase genes in *C. elegans* (Veronico et al., 2001; Zhang et al., 2005b) and is expressed specifically in the pharynx (Fig. 2I and references as above). Secreted chitin lines the lumen walls of the pharynx and the pharyngeal grinder (the worm's teeth). RNAi knockdown of *chs-2* causes animals to arrest as L1 larvae (Zhang et al., 2005b). Expressing *chs-2* under its own promoter fully rescued the *bav-1* food-avoidance phenotype (Fig. 2J). Taken together with my studies of other feeding-defective mutants, these data suggest that *chs-2* mutants are chronically undernourished as a result of defects in pharyngeal function, and that this evokes aversion to the bacteria that the animals are growing on.

Food avoidance is mediated via the sensory neurons AWC and AWB, and jointly by ASJ and ASK

Although *eat-2* and *chs-2* animals accumulated off the bacterial food they nevertheless remain close to it, often forming a ring just outside the bacterial lawn (Fig. 1F). This behavior suggested that the animals retained some long-range attraction to food. By contrast, the increased reversals and turns when chronically underfed worms approached food indicated that some aversive cues from the food dominate in the balance between attraction and avoidance at short range. In *C. elegans*, food-associated cues like odors and tastants are sensed predominantly by sensory neurons in the amphids. To dissect food-avoidance behavior in chronically underfed animals at a sensory level, I constructed double mutants defective in *eat-2* or *chs-2* and in genes required for specific neural functions. Mutants in *tax-4*, encoding an α-subunit of a cGMP-gated ion channel, are defective in the function of 14 neuron pairs (Komatsu et al., 1996). *tax-4* mutations suppressed food avoidance in both *eat-2* and *chs-2* mutant animals (Fig. 3A,B). The *tax-2* gene encodes the β-subunit of the TAX-4-containing cGMP-channel and functions in the same 14 neurons. For *tax-2*, a promoter mutation, *tax-2(p694),* is available that selectively disrupts *tax-2* expression in six of the 14 neurons (Coburn and Bargmann, 1996; Coates and de Bono, 2002; Bretscher et al., 2011). The *tax-2(p694)* mutation failed to suppress *chs-2* food avoidance, suggesting that one or more of the remaining *tax-2*/*tax-4*-expressing neurons promoted this behavior. These include the AWB and AWC olfactory neurons, and the ASG, ASI, ASJ and ASK gustatory neurons. I used cell-specific promoters to express *tax-4* cDNA in these neurons singly or in combination (except ASG, for which no cell-specific promoter has been described), and asked where *tax-4* expression was required to restore food-avoidance behavior to *eat-2;tax-4* mutant animals. *tax-4* expression in AWC neurons almost fully rescued the avoidance phenotype (Fig. 3C). Partial rescue was observed when *tax-4* expression was restored to AWB neurons and there was a strong rescue of the avoidance phenotype when *tax-4* was expressed in a combination of three neurons: ASI, ASJ and ASK with the *gpa-10* and *srg-8* promoters (Fig. 3C). As the *gpa-10* promoter drives expression in ASI and ASJ (and several more non-*tax-4*-expressing neurons (Jansen et al., 1999), I sought to test all neurons individually. There was no rescue of avoidance behavior when *tax-4* was individually expressed in ASI, ASJ or ASK (Fig. 3C,D) or in combinations in ASI and ASJ or ASI and ASK. Food-avoidance behavior was only restored in *eat-2;tax-4* animals with combined expression in ASJ and ASK (Fig. 3D). Similarly, the strong phenotype in *eat-2;tax-4* animals in reducing reversals and turns was restored upon expressing the *tax-4* transgene in AWC or a combination of ASJ and ASK (and ASI) (Fig. 3E). As *tax-4* also regulates food leaving (Milward et al., 2011), the food-leaving behavior of *eat-2;tax-4* was analyzed. In agreement with our previous findings, *tax-4* suppressed *eat-2* food leaving. Partial rescue was observed when *tax-4* expression was restored in the sensory neurons that rescued food avoidance of *eat-2;tax-4* (Fig. 3F), indicating that AWB, AWC and ASJ and ASK likely promote food-leaving behavior (Fig. 3F). Hence, *tax-4* and cGMP signaling promote both food leaving and food avoidance in feeding-defective animals.

To investigate further the involvement of AWB and AWC neurons, I asked whether disrupting *odr-1* or *odr-3* suppressed *eat-2* food avoidance (Fig. 3B). ODR-1 encodes a transmembrane guanylyl cyclase necessary for odorant responses mediated by AWC and AWB neurons (L'Etoile and Bargmann, 2000). ODR-3 encodes a Gα protein and is required in several sensory neurons including AWC (Roayaie et al., 1998; Jansen et al., 1999; Lans et al., 2004). Mutations in *odr-1* or *odr-3* partially suppressed *eat-2* food-avoidance behavior, in agreement with the *tax-4* rescue data. In summary, our results suggest that AWC and AWB olfactory neurons are important for feeding-defective worms to avoid food. A separate set of chemosensory neurons, ASJ and ASK, previously implicated in taste responses, also acts to promote food avoidance.

Regulation of avoidance behavior by HEN-1 and TGF-β/DBL-1 signaling

Food that is attractive to well-fed animals repels chronically underfed animals, suggesting that the animals' perception of food is altered in response to their reduced nutritional state. This altered perception likely involves integration of metabolic state and food cues. HEN-1 is a secreted protein with an LDL-a motif that, together with its putative receptor SCD-2, regulates integration of sensory stimuli in the AIA interneuron (Ishihara et al., 2002; Shinkai et al., 2011). *hen-1* mutant animals fail to integrate conflicting sensory cues and are defective in associative learning (Ishihara et al., 2002). I therefore tested whether HEN-1 function is required for the food-avoidance behavior displayed by chronically underfed animals. *hen-1* suppressed the food-avoidance phenotype of *eat-2* mutants (Fig. 4A), although the mutant animals eventually accumulated off food. This phenotype could be rescued by expressing *hen-1* from its own promoter in *hen-1;eat-2* animals (Fig. 4B). These data indicate that *hen-1* promotes food avoidance in undernourished animals, but that its role is not essential.

Avoidance behavior to palatable food by chronically underfed animals could share cellular and neuronal mechanisms with avoidance behavior to harmful (Pujol et al., 2001; Zhang et al., 2005a; Schulenburg and Ewbank, 2007) or indigestible food (Andrew and Nicholas, 1976; Shtonda and Avery, 2006). Naive *C. elegans* are normally attracted to pathogenic bacteria such as *Serratia marcescens* but learn to avoid the bacteria (Pujol et al., 2001; Zhang et al., 2005a). Similarly, *C. elegans* learn to avoid pathogenic *Pseudomonas aeruginosa* (Zhang et al., 2005a) and it was recently reported this olfactory learning relies on the TGF-β/DBL-1 pathway (Zhang and Zhang, 2012). I therefore analyzed how animals defective in components of the DBL-1 pathway responded to food palatable to wild-type animals. All mutants analyzed in the pathway behave similar to chronically underfed worms (Fig. 4C). Mutants in *dbl-1*, encoding the TGF-β ligand of the Sma/Mad pathway (Suzuki et al., 1999), or in its cognate receptors DAF-4 and SMA-6 (Estevez et al., 1993; Krishna et al., 1999), left and avoided food (Fig. 4C,G). Mutants for the downstream signal transducers SMA-2 (R-Smad), SMA-4 (Co-Smad) (Brenner, 1974; Savage et al., 1996) and the transcription factor SMA-9 (Liang et al., 2003) also avoided food (Fig. 4C,D). Moreover, mutants lacking the Kex-2/subtilisin-like pro-protein convertase KPC-1, predicted to process TGF-β like pro-proteins

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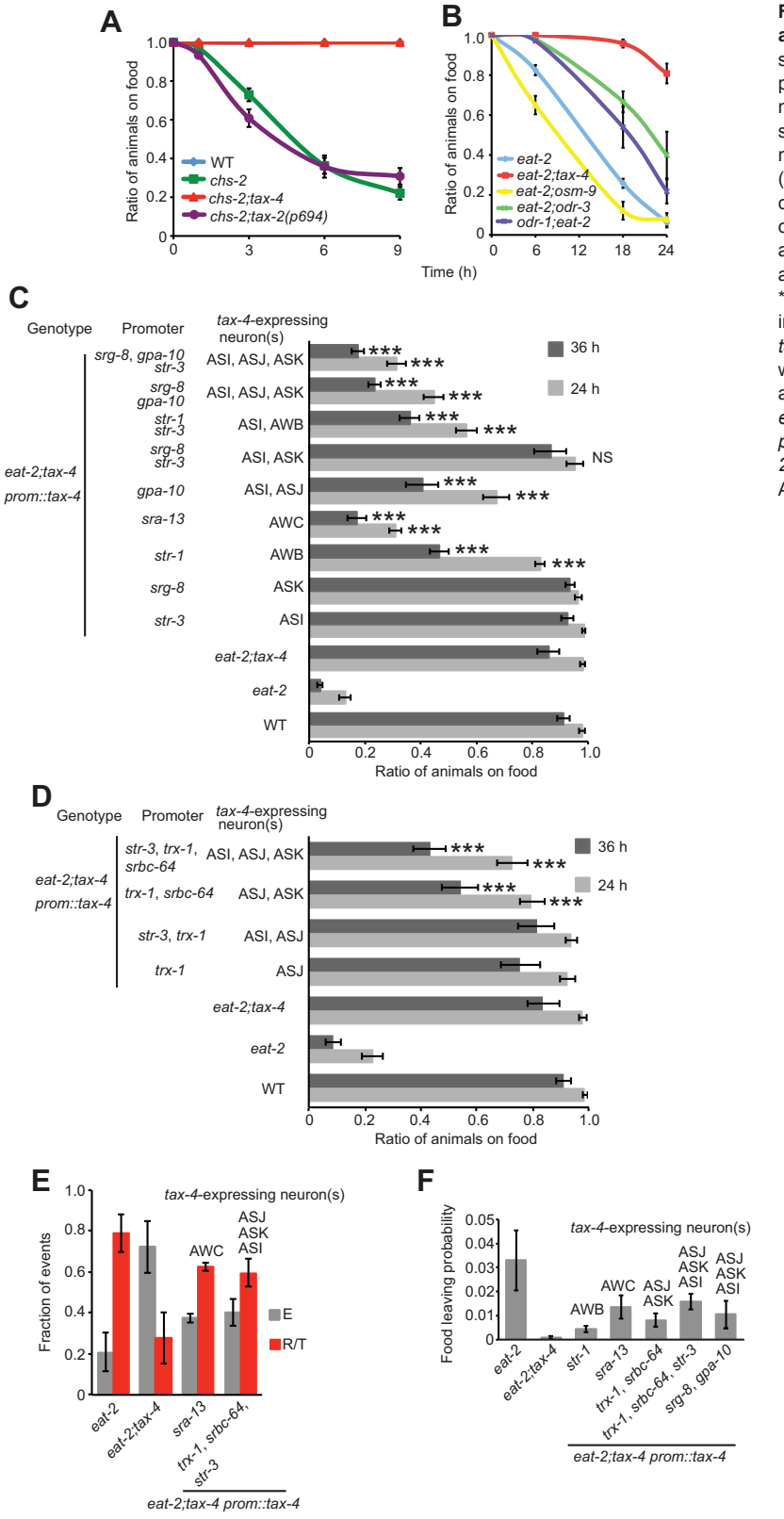

Fig. 3. Food avoidance is regulated by the AWC, AWB, ASJ and ASK neurons. (A) Food avoidance of *chs-2* mutants is suppressed by a *tax-4(null)* mutation but not by *tax-2(p694)* promoter deletion. (B) The food-avoidance phenotype of *eat-2* mutants was fully suppressed by mutations in *tax-4*, and partially suppressed by mutations in *odr-3* and *odr-1*. By contrast, a mutation in *osm-9* enhanced food aversion of *eat-2* animals. (C,D) Food avoidance can be restored to *eat-2;tax-4* animals by cell-specific expression of *tax-4* cDNA in AWC. Expressing *tax-4* cDNA in AWB, or in ASI, ASJ and ASK partially restores food-avoidance behavior in *eat-2;tax-4* animals. Each genotype was assayed at least six times. Error bars represent s.e.m. **P*<0.05, ***P*<0.01, ****P*<0.005, Student's *t*-test. (E) Rescue of food-induced reversals and turns in *eat-4;tax-4* animals expressing *tax-4* cDNA in the indicated neurons (scored manually at 24 h when animals are attempting to re-enter the lawn). Number of attempts scored per genotype: *eat-2*, *N*=68; e*at-2;tax-4*, *N*=62; *eat-2;tax-4 psra-13::tax-4*, *N*=80; *eat-2;tax-4 ptrx-1*, *psrbc-64*, *pstr-3::tax-4*, *N*=79. (F) Food leaving is partially restored in *eat-2;tax-4* animals when *tax-4* is expressed in AWB, AWC and ASJ, ASK and ASI neurons.

(Gómez-Saladin et al., 1997), left and avoided the food strongly. Thus, the DBL-1 pathway acts to suppress food avoidance. This is in contrast to its role in aversive olfactory learning of pathogenic bacteria, where it promotes avoidance of harmful food.

DBL-1 is secreted from the AVA interneurons to promote aversive learning of pathogenic bacteria (Zhang and Zhang, 2012), whereas its receptor SMA-6 acts in the hypodermis and in the chemosensory neuron ASI (Zhang and Zhang, 2012). AVA expression of DBL-1 failed to restore wild-type behavior to *dbl-1* mutants in our assays (Fig. 4E,F). Expressing the SMA-6 receptor, using previously reported strains able to rescue *sma-6* aversive olfactory learning defects (Zhang and Zhang, 2012), in hypodermis (using the *col-12*

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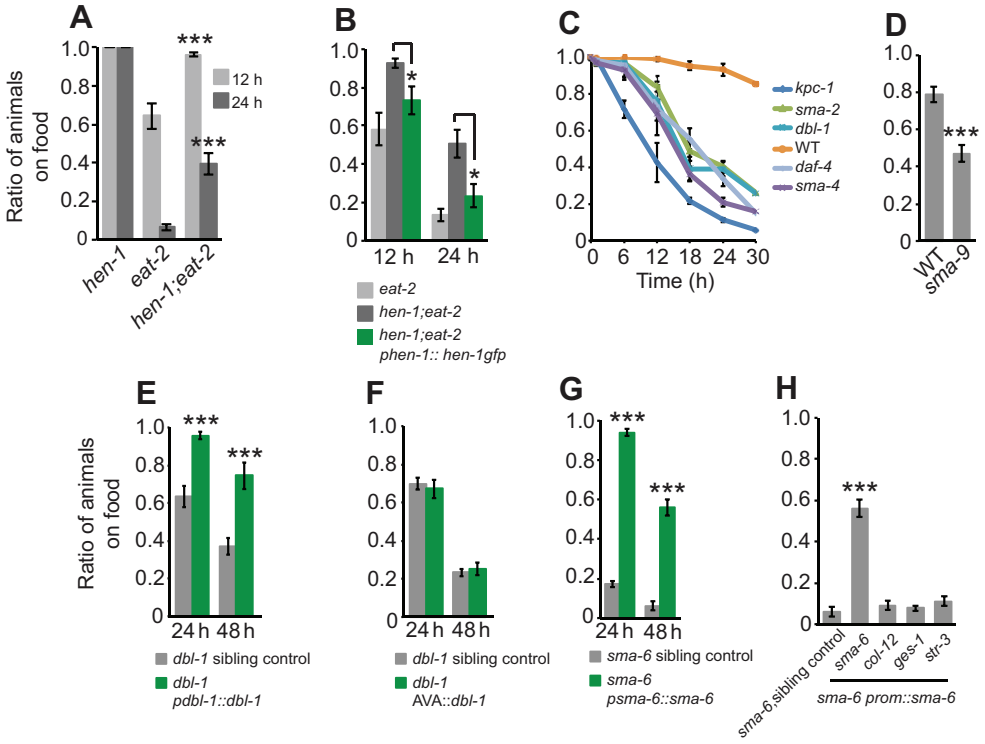

Fig. 4. HEN-1 and the DBL-1/Sma pathway regulate food-avoidance behavior. (A,B) Disrupting *hen-1*, which was previously implicated in sensory integration of attractive and aversive cues, suppressed food aversion in *eat-2* animals (A). A *hen-1* transgene can restore avoidance to *hen-1;eat-2* animals (B). (C) Mutants defective in components of the DBL-1/Sma pathway avoid food. (D) Animals defective in *sma-9*, the downstream transcription factor of the DBL-1/Sma pathway, show a high off-food distribution but a weaker phenotype than other mutants in the pathway. (E) *dbl-1* expression under its own promoter but not in the AVA interneuron restores WT behavior (F). (G) *sma-6* animals show food aversion, which can be rescued by expressing *sma-6* under its own promoter (G), but not under a promoter that express selectively in the hypodermis (*pcol-12*), intestine (*pges-1*) or ASI neurons (*pstr-3*) (H). H shows the 48 h time point. Each genotype was assayed at least six times. Error bars represent s.e.m. **P*<0.05, ***P*<0.01, ****P*<0.005, Student's *t*-test.

promoter) or in ASI neurons (from the *str-3* promoter) did not rescue *sma-6* food-avoidance behavior (Fig. 4G,H). *sma-6* is also expressed in the pharynx (Yoshida et al., 2001), but pharyngeal expression of *sma-6* also failed to restore wild-type behavior (supplementary material Fig. S3). Together, these data suggest that the TGF-β/DBL-1 pathway suppresses food avoidance in this paradigm and imply that this function is distinct from its role in aversive learning.

Avoidance behavior is enhanced by increased population size and suppressed in the absence of functional nutrient sensors

Caenorhabditis elegans feed on the *E.coli* strain OP-50 in the lab and one possibility is that avoidance is a food source peculiarity and not, as hypothesized, linked to the reduced feeding status of the animal. To test this, similar avoidance assays were performed using the *E. coli* strain HB101, described as a good quality food source (Shtonda and Avery, 2006). As predicted, the feeding-defective *eat-2* and *chs-2* mutant animals as well as *sma-6* mutants, but not wild-type animals, avoided HB101 similarly to OP-50 (supplementary material Fig. S4). This further supports the link between feeding status and interpretation of food quality. The value of a food patch is influenced not only by its nutritional quality (perceived or real) but also by the presence of competitors on that patch. Animal responses to food cues are therefore likely to be influenced by the population density on the patch. Consistent with this, previous work has shown that population density modulates olfactory plasticity in *C. elegans* (Yamada et al., 2010). To test the effect of animal number in the avoidance assay, I used both fewer (10 and 20 animals per plate) and more (160 animals per plate) animals than in the standard assay. *chs-2* animals accumulated off food in all conditions, but the response dynamics were accelerated by increasing population size (Fig. 5A).

As increasing population density enhanced avoidance behavior, I tested whether conditioning the food also enhanced avoidance. I conditioned food by incubating 50 animals overnight on a thick food patch, removing these animals and then using the plate as a test plate. Avoidance in *chs-2* mutant animals was strongly enhanced when the bacterial lawn was conditioned (Fig. 5B). This response did not depend on dauer pheromone as *daf-22* mutants, which are unable to produce the potent ascarosides (Golden and Riddle, 1985; Butcher et al., 2009; Pungaliya et al., 2009), conditioned the food to a similar degree as wild-type animals. Taken together, these data suggest that population density modulates the development of bacterial avoidance. Whether this reflects the action of unidentified pheromones or of bacterial metabolites is unclear.

To test whether animals that have already developed food-avoidance behavior responded differently when subsequently presented with fresh food, I compared the behavior of *chs-2* animals pre-incubated on fresh food for 1 h (all animals remain on food, 'on') with that of animals kept on the same lawn overnight (90–100% of animals off food, 'off') when presented with a fresh bacterial lawn. The response to fresh food was indistinguishable across the two conditions (Fig. 5C). These data suggest that fresh food somehow resets the animal's behavioral state.

If the animal's perception of its metabolic state is important for its response to food, interfering with nutrient state sensors should alter food-leaving and food-avoidance behavior. Two major conserved cellular nutrient-sensing pathways are mediated by AMP-activated protein kinase, AMPK, and the TOR-kinase pathway (Lindsley and Rutter, 2004). In *C. elegans*, the *aak-2* gene encodes one of the two catalytic α-subunits of AMPK (Apfeld et al., 2004) and the *let-363* gene encodes TOR-kinase (Long et al., 2002). As mutations in *let-363* cause developmental arrest (Long et al., 2002), I used mutants in its downstream target p70 S6-kinase, *rsks-1* (Pan et al., 2007). The food avoidance of *eat-2* mutant animals was significantly suppressed by a mutation in *aak-2* (Fig. 5D). Similarly, double mutants with *rsks-1* strongly suppressed *chs-2* food avoidance (Fig. 5E). These data suggest that interfering with the animals' ability to assess its nutritional state affects its behavioral responses to food. Food avoidance in response to underfeeding thus

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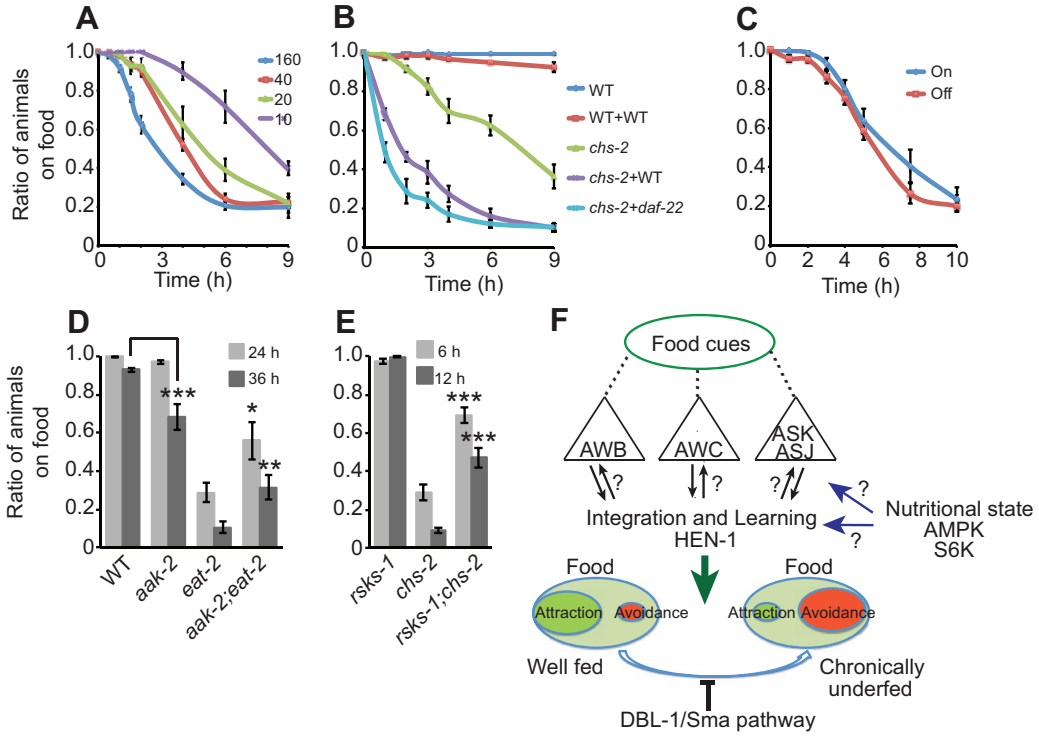

Fig. 5. Food-avoidance behavior is affected by population size and mutations in nutritional state sensors. (A) Food avoidance by *chs-2* mutants is accelerated by increased population density (from 10 to 160 animals per assay plate). (B) Conditioning the food enhances avoidance behavior of *chs-2* mutants. *daf-22* mutants, which are defective in the production of some ascaroside pheromones, including dauer pheromone, condition food similar to WT animals. (C) There is no difference in avoidance whether the *chs-2* animals come from plates with ~100% off-food distribution (pre-incubated overnight on fresh food) versus ~100% on-food distribution (pre-incubated 1 h on fresh food). See Materials and methods for details. (D) Disrupting AMPK, encoded by the *aak-2* gene, suppresses food aversion of *eat-2* mutants. (E) A mutation in S6 kinase, *rsks-1*, suppresses *chs-2* food avoidance. Each genotype was assayed at least six times. Error bars represent s.e.m. **P*<0.05, ***P*<0.01, ****P*<0.005, Student's *t*-test. (F) Model of food aversion in response to chronically reduced feeding/nutritional status. AWC, AWB, ASJ and ASK neurons promote food avoidance in chronically food-deprived animals. AMPK and S6 kinase appear to be involved in sensing nutritional state and promoting food avoidance in chronically underfed animals. The TGF-β/DBL-1 pathway inhibits this food-avoidance behavior via an as yet unknown anatomical location.

relies on intact nutrient sensors, and is strongly affected by population density on the food source.

DISCUSSION

Caenorhabditis elegans that are chronically undernourished because of defects in their alimentary tract develop aversion to a bacterial food source that is attractive to wild-type animals. This plastic response involves animals reversing and turning away from food despite their malnourished state. The behavior involves the AWC and AWB olfactory neurons, and the ASK and ASJ chemosensory neurons, and is also regulated by the nutrient state sensors AMP kinase and S6 kinase.

Avoidance by *C. elegans* of normally attractive food has been reported previously (Melo and Ruvkun, 2012). This study inactivated core cellular processes (e.g. mitochondrial function) using RNAi and it was observed that in many cases animals distributed off food more than wild-type. The authors did not distinguish between food-leaving and food-aversive behavior, although only a small subset of the RNAi-treated animals accumulated off food (i.e. had an aversion score higher than 0.5). These authors suggested this response to be part of a pathogen defense mechanism. Given my results, I suggest that the observed food-leaving/food-aversive behavior induced by RNAi treatment may reflect an altered sense of nutritional state. Consistent with this, some of the RNAi treatments reduced pharyngeal pumping (Melo and Ruvkun, 2012).

While it is known that nutritional state influences foraging, the mechanisms involved are poorly understood. I show here that in *C. elegans*, two different cellular nutrient sensors, the 5′-AMP-activated protein kinase (AMPK) and the ribosomal S6 kinase, a target of TOR kinase (TORC1), promote food avoidance behavior in chronically underfed *C. elegans*. AMP kinase is activated by increases in the intracellular AMP/ATP ratio, a hallmark of metabolic stress. TOR signaling, and therefore S6 kinase activity, is stimulated by intracellular amino acids, and promotes anabolic metabolism. Decreased food avoidance in *C. elegans* AMP kinase mutants could reflect a compromised ability to perceive poor nutritional state. The phenotype of *C. elegans* S6 kinase mutants could reflect reduced energy demand: *rsks-1* mutants grow more slowly and have smaller broods than wild-type animals (Pan et al., 2007). However, given the complex functions of these nutrient sensors, we cannot exclude other or additional explanations. *aak-2* mutant animals exhibit slightly elevated pharyngeal pumping, suggesting increased food intake (Cunningham et al., 2012). Inactivating AMPK in *Drosophila melanogaster* also causes hyperphagia (Johnson et al., 2010). A role for S6 kinase in a different kind of food-choice paradigm has been described in *Drosophila*. Downregulating ribosomal S6 kinase triggers well-fed *D. melanogaster* larvae to exploit hard-to-get food that is normally rejected by well-fed animals but accepted by food-deprived ones (Wu et al., 2005). This phenotype suggests that flies with reduced S6 kinase activity have increased motivated foraging and feeding.

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In adult flies, S6 kinase acts in unidentified neuronal pathways to regulate nutrient preference: downregulating S6-kinase induces a preference for a protein-rich yeast diet (Ribeiro and Dickson, 2010; Vargas et al., 2010). Both AMPK and TOR (mTORC1) regulate food intake in mammals (Pimentel et al., 2013). Activation of TOR kinase in the hypothalamus decreases food intake whilst activation of AMPK increases food intake. The mechanisms by which the AMPK and TOR pathways influence feeding behavior are not fully understood and cell-specific roles remain to be dissected (Kola, 2008).

The neuronal mechanisms promoting food-avoidance behavior in chronically underfed worms are reminiscent of those involved in avoidance of pathogenic food. Both behaviors involve the olfactory neurons AWC and AWB, which respond to changes in odor concentrations via a cGMP signaling cascade (Ha et al., 2010). The AWC neurons normally direct attractive responses, but can in some circumstances mediate repulsive responses (Tsunozaki et al., 2008), as in the underfed animals described here. In well-fed animals, AWC (with ASK) drives area-restricted search behavior (Wakabayashi et al., 2004; Gray et al., 2005), which allows animals to remain in an area where they last found food. The AWB neurons mediate avoidance of noxious odors (Bargmann, 2006) and direct avoidance of certain bacterial products (Pradel et al., 2007). However, there are some differences between food aversion to normally palatable food and aversive learning. Animals with defects in the TGF-β/DBL-1 pathway fail to learn to avoid pathogenic bacteria (Zhang and Zhang, 2012) and have reduced naive preference. I find that animals defective in DBL-1 and its downstream signaling effectors behave very similar to chronically underfed animals: they leave and avoid good food, indicating that the DBL-1 pathway acts to suppress food avoidance. This behavior could be connected to defects in naive preference as it is clearly distinct from aversive learning given neither the ligand DBL-1 nor the receptor SMA-6 act in the same cells and tissues as in learning. My data rather suggest an endocrine signaling mechanism and it will be interesting to identify the cellular focus where *dbl-1* and *sma-6* function.

The other set of neurons we define that promotes food-avoidance behavior includes the ciliated gustatory neurons ASJ and ASK. Neural imaging shows that ASK neurons respond to food-conditioned media by tonically reducing their cytosolic Ca^{2+} levels (Wakabayashi et al., 2004). Ca^{2+} levels in ASK are transiently inhibited by ascarosides (Macosko et al., 2009), pheromones that drive dauer development (Golden and Riddle, 1982; Jeong et al., 2005) and mediate male attraction to hermaphrodites (Srinivasan et al., 2008; Jang et al., 2012). Like ASK, ASJ neurons transmit pheromone responses and promote dauer development (Schackwitz et al., 1996). ASK is a major post-synaptic target of ASJ.

Is food avoidance in chronically underfed animals a learnt response? Disrupting *hen-1*, which has been implicated in learning and integration of antagonistic sensory stimuli suppresses the aversion phenotype of *eat-2* but it is not required as the animals eventually accumulate off food. The *Drosophila* homolog of the *hen-1* receptor, Alk, has been proposed to spare the CNS during nutrient restriction by activating PI3-kinase (normally activated by the insulin receptor) (Cheng et al., 2011) as well as being implicated in learning (Gouzi et al., 2011). This suggests a wider role for this pathway. It is possible that *C. elegans* HEN-1 signaling also has dual roles.

Food avoidance is strongly affected by population density and this effect seems not to be mediated via dauer pheromones. Interestingly, it was recently reported that survival of L1 larvae during starvation is density dependent, and that this effect is not mediated by ascarosides/dauer pheromone but rather by an unknown starvation signal (Artyukhin et al., 2013). It is possible that this proposed starvation signal also promotes food leaving and avoidance in underfed adult animals. My preliminary data suggest that even a very distantly related species, *Pristionchus pacificus*, is able to condition food to enhance food avoidance of *chs-2* (B.O., unpublished) which could further support my finding that dauer pheromone is not responsible for the conditioning effect. It will be interesting to find the molecular identity of this signal. Exudates from *C. elegans* have been reported to contain a large variety of compounds, some of which are known to be aversive to *C. elegans* (Ward, 1973; Kaplan et al., 2009).

In summary, *C. elegans* that are chronically underfed as a result of defects in their alimentary tract avoid high quality and abundant food. The behavior is dependent on cGMP signaling via TAX-4 in the odor-sensing neurons AWC and AWB. This illustrates that AWC can support aversive behavior in a wider context than previously recognized. Sensory input from the chemosensory ASJ and ASK neurons also promotes food-avoidance behavior. As population density affects avoidance, it will be interesting to find how *C. elegans* interpret population density and whether this is mediated via ASJ and/or ASK. Avoidance behavior is suppressed when metabolic state sensing is disrupted, when animals are defective in sensory integration and in well-fed animals through the DBL-1 pathway. This study illustrates how long-term feeding experiences can alter foraging and could potentially lead to insights into eating disorders such as anorexia nervosa.

MATERIALS AND METHODS

Materials

For strains and rescue constructs see supplementary material Table S1 and below.

Behavioral assays

For food-avoidance assays, regular NGM plates were seeded 2 days prior to assay with 200 µl of OP50 (CGC) grown overnight in 2×YT. The rich 2×YT medium allows growth of a thick food patch. Forty young adults were picked (as much as possible from outside food to avoid transfer of food between the growth plate and the assay plate) to each assay plate and placed in the center of the food lawn. The animals were left to forage and at each time point the ratio of worms on food versus total worms was calculated. Feeding-defective assays were performed similar to methods described previously (Milward et al., 2011) with the exception that the worms were left to settle for 1 h and then assayed immediately (i.e. in high food condition). Food responses (reversals and turns) were scored manually at 24 h on low peptone plates (to increase leaving events of N2 wild-type animals). Low peptone plates contain 5% of the bactopeptone found in NGM medium (Milward et al., 2011). Animals approaching the bacterial lawn from off food either entered food (E), or hesitated, reversed or turned away from food to remain off food (R/T). To condition the food, 50 adults of indicated genotype were left on food overnight and subsequently removed to generate a conditioned assay plate.

For the HB101 experiment in supplementary material Fig. S4, the animals were grown on OP-50 and assayed on a 2 day old lawn on HB101 in 2×YT.

Mapping and cloning of *bav-1*

Linkage analysis and SNP mapping located *bav-1* to the left arm of chromosome II, left of SNP, F46F5. Recombination suppression in this genomic area precluded finer mapping. This left a region of 809 kb where *bav-1* could be located. Genomic DNA was prepared from *bav-1* mutants and sequenced on an Illumina GAIIx machine to ~16× coverage. The bioinformatics analysis of the sequence data identified two polymorphisms in non-intergenic regions within these 809 kbp. These changes were re-sequenced with standard dideoxy sequencing and one change was

confirmed. The change was a G→A point mutation at position 3846 of the *chs-2* gene (www.wormbase.org). This point mutation changes the 3′ splice acceptor site of intron 6 from CAG to CAA. For rescue experiments, 2.0 kb of the *chs-2* promoter was cloned (by nested PCR) into a pDEST Gateway vector containing the 3′ UTR of the *unc-54* gene (5′ primer including an *Nhe* I site: ATATGCTAGCTGAGAATTGCTTGTTTGCG; 3′ primer including a *Xho* I site: ATATCTCGAGGTTTAATAGAAGCTCAGAAATT) and the *chs-2* gene was amplified from genomic DNA with nested PCR (5′ primer including a *Sac* I site: ATCGGAGCTCAAACATGATGAACA-CATTGGACCATC; 3′ primer including a *Kpn*I site: ATCGGGTACC-TTAAAATCTTCTCAGCTCCACGTC) and ligated into a Gateway pENTRY vector containing an intercistronic region (from the *gpd-2 gpd-3* operon) followed by GFP upstream of the attL2 site [both the pDEST and the pENTRY vectors are described elsewhere (Coates and de Bono, 2002)]. A pEXP clone was generated by LR-clonase recombination (Invitrogen). The construct was injected into *bav-1* mutants (outcrossed three times) and GFP expression in the pharynx as well as rescue of the behavioral phenotype was observed.

Transgenic strain generation

The *tax-4* expression constructs were generated using either two-way gateway or multisite gateway cloning.

Primers for *psra-13* pDEST cloning

A 918 bp fragment upstream of the *sra-13* gene, two nucleotides upstream of the ATG was cloned as an *Nhe* 1 and *Xho* 1 fragment into a modified pDEST vector containing the 3′ UTR of unc-54 (primers: 5′-TAAGCTAGCGAATTCGTGAAAAAGTCCACCG-3′ and reverse 5′-TTACTCGAGTAGTGGAAATATCTGAGTTAGTTG-3′).

The *tax-4* pENTRY clone

The *ptrx-1::tax-4* construct was a gift from C. Chen and K. E. Busch. A 1 kb fragment of the trx-1 promoter had been amplified with the following primers: forward GGGGACAACTTTGTATAGAAAAGTTG(=attB4)-agaa-tggatacctgatcattc and reverse GGGGACTGCTTTTTTGTACAAACT-TG(attB1r)-gatgaaatacaagtgtagaaaattc.

The *psrbc-64::tax-4* construct was a kind gift from C. Chen and M. de Bono. A 1.8 kb fragment of the *srbc-64* promoter had been amplified with the following primers: forward GGGGACAACTTTGTATAGAAAA-GTTG(=attB4)-gttttctaaaaatgagatattactagtggtttgattgctaaacc and reverse GGGGACTGCTTTTTTGTACAAACTTG(attB1r)-cagactgtgacaagaaaact-gaaatatcaaaaaaaaaagg.

All *tax-4* rescue constructs were injected at 40–50 ng μl^{-1}. Three independent lines were tested for all rescue experiments.

For *hen-1* rescue, *hen-1;eat-2* animals were injected with a genomic *hen-1gfp* construct (a gift from T. Ishihara).

Dye filling

DiO labeling was done in the presence of food (1:200 dilution of stock solution 2 mg ml^{-1} in DMF) for 16–18 h rotating at room temperature. After labeling, the worms were moved to freshly seeded plates and mounted and examined after 3 h.

Chemoattraction/avoidance and osmotic avoidance tests

Attraction to NaCl, benzaldehyde and diacetyl (1:1000) and avoidance of 1-octanol, 2-nonanone and 8 mol l^{-1} glycerol was essentially done as described elsewhere (Hart, 2006).

Fat staining

Fixed worms were stained with Nile Red [essentially as described previously (Brooks et al., 2009)] with the exception of the fixation, which was done in 1% paraformaldehyde and three cycles of freeze-thawing.

Acknowledgements

I am grateful to Howard Baylis, Mario de Bono and members of the de Bono lab for reviewing the manuscript. Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). I also want to thank everyone who generously provided strains and constructs: Yun Zhang, Simon Tuck, Takeshi Ishihara, Changchun Chen and Mario de Bono. I also would like to thank Ralf Schnabel for depositing the strain GE337 with CGC and noting its bacterial avoidance behavior. Finally, I thank Stefanie Reichelt for help with microscopy and Simon Tuck for a short visit to his lab to learn fat staining.

Competing interests

The author declares no competing financial interests.

Funding

This work was funded by the Medical Research Council, United Kingdom. Deposited in PMC for immediate release.

Supplementary material

Supplementary material available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.099929/-/DC1

References

Andrew, P. A. and Nicholas, W. L. (1976). Effect of bacteria on dispersal of *Caenorhabditis elegans* (Rhabditidae). *Nematologica* **22**, 451-461.
Apfeld, J., O'Connor, G., McDonagh, T., DiStefano, P. S. and Curtis, R. (2004). The AMP-activated protein kinase AAK-2 links energy levels and insulin-like signals to lifespan in *C. elegans*. *Genes Dev.* **18**, 3004-3009.
Artyukhin, A. B., Schroeder, F. C. and Avery, L. (2013). Density dependence in *Caenorhabditis* larval starvation. *Sci. Rep.* **3**, 2777.
Avery, L. (1993). The genetics of feeding in *Caenorhabditis elegans*. *Genetics* **133**, 897-917.
Bargmann, C. I. (2006). *Chemosensation in C. elegans*. The C. elegans Research Community, WormBook. http://www.wormbook.org.
Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71-94.
Bretscher, A. J., Kodama-Namba, E., Busch, K. E., Murphy, R. J., Soltesz, Z., Laurent, P. and de Bono, M. (2011). Temperature, oxygen, and salt-sensing neurons in *C. elegans* are carbon dioxide sensors that control avoidance behavior. *Neuron* **69**, 1099-1113.
Brooks, K. K., Liang, B. and Watts, J. L. (2009). The influence of bacterial diet on fat storage in *C.elegans*. *PLoS ONE* **4**, e7545.
Butcher, R. A., Ragains, J. R., Li, W., Ruvkun, G., Clardy, J. and Mak, H. Y. (2009). Biosynthesis of the *Caenorhabditis elegans* dauer pheromone. *Proc. Natl. Acad. Sci. USA* **106**, 1875-1879.
Cheng, L. Y., Bailey, A. P., Leevers, S. J., Ragan, T. J., Driscoll, P. C. and Gould, A. P. (2011). Anaplastic lymphoma kinase spares organ growth during nutrient restriction in *Drosophila*. *Cell* **146**, 435-447.
Coates, J. C. and de Bono, M. (2002). Antagonistic pathways in neurons exposed to body fluid regulate social feeding in *Caenorhabditis elegans*. *Nature* **419**, 925-929.
Coburn, C. M. and Bargmann, C. I. (1996). A putative cyclic nucleotide-gated channel is required for sensory development and function in *C. elegans*. *Neuron* **17**, 695-706.
Cunningham, K. A., Hua, Z., Srinivasan, S., Liu, J., Lee, B. H., Edwards, R. H. and Ashrafi, K. (2012). AMP-activated kinase links serotonergic signaling to glutamate release for regulation of feeding behavior in *C. elegans*. *Cell Metab.* **16**, 113-121.
Davis, M. W., Somerville, D., Lee, R. Y., Lockery, S., Avery, L. and Fambrough, D. M. (1995). Mutations in the *Caenorhabditis elegans* Na,K-ATPase alpha-subunit gene, eat-6, disrupt excitable cell function. *J. Neurosci.* **15**, 8408-8418.
Estevez, M., Attisano, L., Wrana, J. L., Albert, P. S., Massagué, J. and Riddle, D. L. (1993). The daf-4 gene encodes a bone morphogenetic protein receptor controlling *C. elegans* dauer larva development. *Nature* **365**, 644-649.
Golden, J. W. and Riddle, D. L. (1982). A pheromone influences larval development in the nematode *Caenorhabditis elegans*. *Science* **218**, 578-580.
Golden, J. W. and Riddle, D. L. (1985). A gene affecting production of the *Caenorhabditis elegans* dauer-inducing pheromone. *Mol. Gen. Genet.* **198**, 534-536.
Gómez-Saladín, E., Luebke, A. E., Wilson, D. L. and Dickerson, I. M. (1997). Isolation of a cDNA encoding a Kex2-like endoprotease with homology to furin from the nematode *Caenorhabditis elegans*. *DNA Cell Biol.* **16**, 663-669.
Gouzi, J. Y., Moressis, A., Walker, J. A., Apostolopoulou, A. A., Palmer, R. H., Bernards, A. and Skoulakis, E. M. (2011). The receptor tyrosine kinase Alk controls neurofibromin functions in *Drosophila* growth and learning. *PLoS Genet.* **7**, e1002281.
Gray, J. M., Hill, J. J. and Bargmann, C. I. (2005). A circuit for navigation in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **102**, 3184-3191.
Ha, H. I., Hendricks, M., Shen, Y., Gabel, C. V., Fang-Yen, C., Qin, Y., Colón-Ramos, D., Shen, K., Samuel, A. D. and Zhang, Y. (2010). Functional organization of a neural network for aversive olfactory learning in *Caenorhabditis elegans*. *Neuron* **68**, 1173-1186.
Hart, A. C., Behavior (2006). *WormBook* (ed. The *C. elegans* Research Community, WormBook) doi/10.1895/wormbook.1.87.1, http://www.wormbook.org.
Ishihara, T., Iino, Y., Mohri, A., Mori, I., Gengyo-Ando, K., Mitani, S. and Katsura, I. (2002). HEN-1, a secretory protein with an LDL receptor motif, regulates sensory integration and learning in *Caenorhabditis elegans*. *Cell* **109**, 639-649.
Jang, H., Kim, K., Neal, S. J., Macosko, E., Kim, D., Butcher, R. A., Zeiger, D. M., Bargmann, C. I. and Sengupta, P. (2012). Neuromodulatory state and sex specify alternative behaviors through antagonistic synaptic pathways in *C. elegans*. *Neuron* **75**, 585-592.
Jansen, G., Thijssen, K. L., Werner, P., van der Horst, M., Hazendonk, E. and Plasterk, R. H. (1999). The complete family of genes encoding G proteins of *Caenorhabditis elegans*. *Nat. Genet.* **21**, 414-419.

Jeong, P. Y., Jung, M., Yim, Y. H., Kim, H., Park, M., Hong, E., Lee, W., Kim, Y. H., Kim, K. and Paik, Y. K. (2005). Chemical structure and biological activity of the *Caenorhabditis elegans* dauer-inducing pheromone. *Nature* **433**, 541-545.

Johnson, E. C., Kazgan, N., Bretz, C. A., Forsberg, L. J., Hector, C. E., Worthen, R. J., Onyenwoke, R. and Brenman, J. E. (2010). Altered metabolism and persistent starvation behaviors caused by reduced AMPK function in *Drosophila*. *PLoS ONE* **5**, e12799.

Kaplan, F., Badri, D. V., Zachariah, C., Ajredini, R., Sandoval, F. J., Roje, S., Levine, L. H., Zhang, F., Robinette, S. L., Alborn, H. T. et al. (2009). Bacterial attraction and quorum sensing inhibition in *Caenorhabditis elegans* exudates. *J. Chem. Ecol.* **35**, 878-892.

Kola, B. (2008). Role of AMP-activated protein kinase in the control of appetite. *J. Neuroendocrinol.* **20**, 942-951.

Komatsu, H., Mori, I., Rhee, J. S., Akaike, N. and Ohshima, Y. (1996). Mutations in a cyclic nucleotide-gated channel lead to abnormal thermosensation and chemosensation in *C. elegans*. *Neuron* **17**, 707-718.

Krishna, S., Maduzia, L. L. and Padgett, R. W. (1999). Specificity of TGFbeta signaling is conferred by distinct type I receptors and their associated SMAD proteins in *Caenorhabditis elegans*. *Development* **126**, 251-260.

L'Etoile, N. D. and Bargmann, C. I. (2000). Olfaction and odor discrimination are mediated by the *C. elegans* guanylyl cyclase ODR-1. *Neuron* **25**, 575-586.

Lans, H., Rademakers, S. and Jansen, G. (2004). A network of stimulatory and inhibitory Galpha-subunits regulates olfaction in *Caenorhabditis elegans*. *Genetics* **167**, 1677-1687.

Liang, J., Lints, R., Foehr, M. L., Tokarz, R., Yu, L., Emmons, S. W., Liu, J. and Savage-Dunn, C. (2003). The *Caenorhabditis elegans* schnurri homolog sma-9 mediates stage- and cell type-specific responses to DBL-1 BMP-related signaling. *Development* **130**, 6453-6464.

Lindsley, J. E. and Rutter, J. (2004). Nutrient sensing and metabolic decisions. *Comp. Biochem. Physiol.* **139B**, 543-559.

Long, X., Spycher, C., Han, Z. S., Rose, A. M., Muller, F. and Avruch, J. (2002). TOR deficiency in *C. elegans* causes developmental arrest and intestinal atrophy by inhibition of mRNA translation. *Curr. Biol.* **12**, 1448-1461.

Macosko, E. Z., Pokala, N., Feinberg, E. H., Chalasani, S. H., Butcher, R. A., Clardy, J. and Bargmann, C. I. (2009). A hub-and-spoke circuit drives pheromone attraction and social behaviour in *C. elegans*. *Nature* **458**, 1171-1175.

McKay, J. P., Raizen, D. M., Gottschalk, A., Schafer, W. R. and Avery, L. (2004). eat-2 and eat-18 are required for nicotinic neurotransmission in the *Caenorhabditis elegans* pharynx. *Genetics* **166**, 161-169.

Melo, J. A. and Ruvkun, G. (2012). Inactivation of conserved *C. elegans* genes engages pathogen- and xenobiotic-associated defenses. *Cell* **149**, 452-466.

Milward, K., Busch, K. E., Murphy, R. J., de Bono, M. and Olofsson, B. (2011). Neuronal and molecular substrates for optimal foraging in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **108**, 20672-20677.

Mörck, C., Rauthan, M., Wågberg, F. and Pilon, M. (2004). pha-2 encodes the *C. elegans* ortholog of the homeodomain protein HEX and is required for the formation of the pharyngeal isthmus. *Dev. Biol.* **272**, 403-418.

Pan, K. Z., Palter, J. E., Rogers, A. N., Olsen, A., Chen, D., Lithgow, G. J. and Kapahi, P. (2007). Inhibition of mRNA translation extends lifespan in *Caenorhabditis elegans*. *Aging Cell* **6**, 111-119.

Pimentel, G. D., Ropelle, E. R., Rocha, G. Z. and Carvalheira, J. B. (2013). The role of neuronal AMPK as a mediator of nutritional regulation of food intake and energy homeostasis. *Metabolism* **62**, 171-178.

Pradel, E., Zhang, Y., Pujol, N., Matsuyama, T., Bargmann, C. I. and Ewbank, J. J. (2007). Detection and avoidance of a natural product from the pathogenic bacterium *Serratia marcescens* by *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **104**, 2295-2300.

Provenza, F. D. (1996). Acquired aversions as the basis for varied diets of ruminants foraging on rangelands. *J. Anim. Sci.* **74**, 2010-2020.

Pujol, N., Link, E. M., Liu, L. X., Kurz, C. L., Alloing, G., Tan, M. W., Ray, K. P., Solari, R., Johnson, C. D. and Ewbank, J. J. (2001). A reverse genetic analysis of components of the Toll signaling pathway in *Caenorhabditis elegans*. *Curr. Biol.* **11**, 809-821.

Pungaliya, C., Srinivasan, J., Fox, B. W., Malik, R. U., Ludewig, A. H., Sternberg, P. W. and Schroeder, F. C. (2009). A shortcut to identifying small molecule signals that regulate behavior and development in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **106**, 7708-7713.

Ribeiro, C. and Dickson, B. J. (2010). Sex peptide receptor and neuronal TOR/S6K signaling modulate nutrient balancing in *Drosophila*. *Curr. Biol.* **20**, 1000-1005.

Roayaie, K., Crump, J. G., Sagasti, A. and Bargmann, C. I. (1998). The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in *C. elegans* olfactory neurons. *Neuron* **20**, 55-67.

Savage, C., Das, P., Finelli, A. L., Townsend, S. R., Sun, C. Y., Baird, S. E. and Padgett, R. W. (1996). *Caenorhabditis elegans* genes sma-2, sma-3, and sma-4 define a conserved family of transforming growth factor beta pathway components. *Proc. Natl. Acad. Sci. USA* **93**, 790-794.

Schackwitz, W. S., Inoue, T. and Thomas, J. H. (1996). Chemosensory neurons function in parallel to mediate a pheromone response in *C. elegans*. *Neuron* **17**, 719-728.

Schulenburg, H. and Ewbank, J. J. (2007). The genetics of pathogen avoidance in *Caenorhabditis elegans*. *Mol. Microbiol.* **66**, 563-570.

Shinkai, Y., Yamamoto, Y., Fujiwara, M., Tabata, T., Murayama, T., Hirotsu, T., Ikeda, D. D., Tsunozaki, M., Iino, Y., Bargmann, C. I. et al. (2011). Behavioral choice between conflicting alternatives is regulated by a receptor guanylyl cyclase, GCY-28, and a receptor tyrosine kinase, SCD-2, in AIA interneurons of *Caenorhabditis elegans*. *J. Neurosci.* **31**, 3007-3015.

Shtonda, B. B. and Avery, L. (2006). Dietary choice behavior in *Caenorhabditis elegans*. *J. Exp. Biol.* **209**, 89-102.

Srinivasan, J., Kaplan, F., Ajredini, R., Zachariah, C., Alborn, H. T., Teal, P. E., Malik, R. U., Edison, A. S., Sternberg, P. W. and Schroeder, F. C. (2008). A blend of small molecules regulates both mating and development in *Caenorhabditis elegans*. *Nature* **454**, 1115-1118.

Suzuki, Y., Yandell, M. D., Roy, P. J., Krishna, S., Savage-Dunn, C., Ross, R. M., Padgett, R. W. and Wood, W. B. (1999). A BMP homolog acts as a dose-dependent regulator of body size and male tail patterning in *Caenorhabditis elegans*. *Development* **126**, 241-250.

Tsunozaki, M., Chalasani, S. H. and Bargmann, C. I. (2008). A behavioral switch: cGMP and PKC signaling in olfactory neurons reverses odor preference in *C. elegans*. *Neuron* **59**, 959-971.

Vargas, M. A., Luo, N., Yamaguchi, A. and Kapahi, P. (2010). A role for S6 kinase and serotonin in postmating dietary switch and balance of nutrients in *D. melanogaster*. *Curr. Biol.* **20**, 1006-1011.

Veronico, P., Gray, L. J., Jones, J. T., Bazzicalupo, P., Arbucci, S., Cortese, M. R., Di Vito, M. and De Giorgi, C. (2001). Nematode chitin synthases: gene structure, expression and function in *Caenorhabditis elegans* and the plant parasitic nematode *Meloidogyne artiellia*. *Mol. Gen. Genet.* **266**, 28-34.

Wakabayashi, T., Kitagawa, I. and Shingai, R. (2004). Neurons regulating the duration of forward locomotion in *Caenorhabditis elegans*. *Neurosci. Res.* **50**, 103-111.

Ward, S. (1973). Chemotaxis by the nematode *Caenorhabditis elegans*: identification of attractants and analysis of the response by use of mutants. *Proc. Natl. Acad. Sci. USA* **70**, 817-821.

Wu, Q., Zhang, Y., Xu, J. and Shen, P. (2005). Regulation of hunger-driven behaviors by neural ribosomal S6 kinase in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **102**, 13289-13294.

Yamada, K., Hirotsu, T., Matsuki, M., Butcher, R. A., Tomioka, M., Ishihara, T., Clardy, J., Kunitomo, H. and Iino, Y. (2010). Olfactory plasticity is regulated by pheromonal signaling in *Caenorhabditis elegans*. *Science* **329**, 1647-1650.

Yoshida, S., Morita, K., Mochii, M. and Ueno, N. (2001). Hypodermal expression of Caenorhabditis elegans TGF-beta type I receptor SMA-6 is essential for the growth and maintenance of body length. *Dev. Biol.* **240**, 32-45.

Zhang, X. and Zhang, Y. (2012). DBL-1, a TGF-β, is essential for *Caenorhabditis elegans* aversive olfactory learning. *Proc. Natl. Acad. Sci. USA* **109**, 17081-17086.

Zhang, Y., Lu, H. and Bargmann, C. I. (2005a). Pathogenic bacteria induce aversive olfactory learning in *Caenorhabditis elegans*. *Nature* **438**, 179-184.

Zhang, Y., Foster, J. M., Nelson, L. S., Ma, D. and Carlow, C. K. (2005b). The chitin synthase genes chs-1 and chs-2 are essential for *C. elegans* development and responsible for chitin deposition in the eggshell and pharynx, respectively. *Dev. Biol.* **285**, 330-339.