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Food sensitizes *C. elegans* avoidance behaviours through acute dopamine signalling

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Many behavioural states are modulated by food availability and nutritional status. Here, we report that in *Caenorhabditis elegans*, the presence of an external food source enhances avoidance responses to soluble repellents sensed by the polymodal ASH neurons. This enhancement requires dopamine signalling and is mimicked by exogenous dopamine. Food modulation is dependent on the mechanosensory cilia of the dopaminergic neurons, indicating that dopamine is released in response to sensation of bacteria. Activation of the dopamine neurons leads within seconds to a transient state of increased sensory acuity. *In vivo* imaging experiments indicate that this dopamine-dependent sensitization results in part from modality-specific increases in the magnitude and duration of gustatory responses in the ASH neurons. The D1-like dopamine receptor DOP-4 acts cell autonomously in ASH to mediate effects on response magnitude. Thus, dopamine functions as a direct signal of the presence of food to control context-dependent behavioural states.
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Introduction

Sensory systems in both simple and complex animals are not static, but can switch between alternative behavioural states. This plasticity allows animals to adjust their behaviours in response to changes in the internal or external environment, such as feeding state, nutritional status, sleep deprivation and stress. To switch between behavioural states, the nervous system utilizes neuromodulators such as biogenic amines and neuropeptides. Neuromodulators act by altering the properties of specific neurons, resulting in changes in information processing and behavioural responses.

The nematode *Caenorhabditis elegans* offers a useful system to study neuromodulation at the behavioural, cellular and molecular levels. *C. elegans* has a compact nervous system consisting of 302 neurons, of which 32 are chemosensory and 28 are mechanosensory (Bargmann and Mori, 1997). Despite this small number, nematodes can sense a diverse variety of chemical and mechanical stimuli and generate relatively complex behaviours. *C. elegans* uses a large number of neuromodulators, including the monoamines serotonin, dopamine, octopamine and tyramine, as well as many neuropeptides (Rand and Nonet, 1997). In addition, a range of sophisticated genetic tools exists for studying behavioural states at the molecular level.

One particularly important factor that guides behaviour is feeding state. In the laboratory, *C. elegans* is grown using the *E. coli* strain OP50 as a food source, and various types of behaviours have been shown to be affected by the presence or absence of bacterial food. Specifically, the presence of food stimulates feeding (Avery and Horvitz, 1990) and egg laying (Trent *et al*, 1983), slows the rate of locomotion (Sawin *et al*, 2000), and modifies responses to sensory stimuli (Saeki *et al*, 2001; Chao *et al*, 2004; Hilliard *et al*, 2005; Mohri *et al*, 2005; Harris *et al*, 2009). These behavioural changes depend on a variety of neuromodulators, in particular, monoamines. For example, serotonin has been implicated in the food-dependent modulation of feeding (Horvitz *et al*, 1982) and locomotion (Sawin *et al*, 2000); tyramine affects foraging and the control of egg laying (Rex *et al*, 2004; Alkema *et al*, 2005); and octopamine is important in starvation-induced changes in gene expression (Suo *et al*, 2006). Neuropeptides also appear to be involved in food modulation of behaviour; for example, the *flp-1* neuropeptide gene is required for the stimulation of egg laying by bacteria (Waggoner *et al*, 2000), and insulin-like peptides are important for some forms of food-dependent sensory plasticity (Kodama *et al*, 2006; Tomioka *et al*, 2006). For all these examples, however, it remains unclear whether these modulators are directly mobilized by the presence or absence of food, and if so what the neural and molecular mechanisms might be.

Another molecule strongly implicated as a direct signal of food in *C. elegans* is dopamine. Dopamine is required for the two effects of food on locomotion: a decrease in locomotion speed when worms encounter a food source (Sawin *et al*, 2000) and an increase in turn frequency when worms leave food (Hills *et al*, 2004). Dopamine is also required for the suppression of CREB-dependent gene expression in well-fed animals (Suo *et al*, 2009), as well as for the slowing of touch habituation on food (Sanyal *et al*, 2004; Kindt *et al*, 2007). There are only eight dopaminergic neurons in *C. elegans*: four CEPs in the nose, two ADEs in the head and two PDEs in the body (Sulston *et al*, 1975). All are ciliated neurons with putative mechanosensory dendrites, and the CEPs in particular have been shown to respond directly to mechanical stimuli (Kindt *et al*, 2007; Kang *et al*, 2010). This and other evidence suggest that the dopaminergic neurons directly sense the presence of bacteria using a mechanosensory modality.

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In this study, we identify a new dopamine-mediated effect of feeding state on *C. elegans* behaviour. We find that responses to soluble repellents are enhanced in the presence of food, an effect dependent on direct food sensing by the dopamine neurons. Optical activation of the dopaminergic neurons mimics this effect within seconds, indicating that dopamine acutely modulates food-regulated behaviours. The enhancement of repellent responses is mediated in part by DOP-4, a D1-like dopamine receptor that functions in the ASH nociceptors to increase the magnitude of sensory responses. These results reveal that dopamine acts to signal the presence of food and to modify sensory perception in response to feeding state.

Results

Food enhances avoidance responses in ASH neurons

To investigate the effect of feeding state on avoidance responses, we tested how food affects behavioural responses to the soluble repellents copper, primaquine and glycerol. We measured the avoidance behaviour by applying a drop of repellent-containing solution to freely moving animals and scoring repellent-evoked reversals, an assay known as the drop test (Hilliard *et al*, 2002). We observed that wild-type animals showed significantly stronger avoidance of 2 mM $CuCl_2$ in the presence of food than in the absence of food (Figure 1A). Likewise, we observed a significant increase in escape behaviour in response to glycerol (0.5 M) and primaquine (10 mM) in the presence of food compared with that in food-free conditions (Figure 1A). These results indicate that food enhances avoidance of soluble repellents in wild-type animals.

Avoidance of copper, glycerol and primaquine is mainly mediated by the nociceptive neuron ASH (Bargmann *et al*, 1990; Sambongi *et al*, 1999; Hilliard *et al*, 2004). Transient activation of ASH generates more prolonged activation of the backward command interneurons, which evoke escape responses (reversals) by triggering a transition between backward and forward locomotion (Faumont and Lockery, 2006; Guo *et al*, 2009). In principle, the presence of food could enhance avoidance of these compounds by increasing the sensitivity or excitability of the ASH neurons themselves; alternatively, the effect could be the result of changes elsewhere in the escape circuit. To investigate these possibilities, we used calcium imaging to measure repellent-evoked neuronal activity in ASH on- and off-food. We found that in the presence of food, the magnitude of the neuronal responses to copper and glycerol was increased (Figure 1B–D). The presence of food also increased the duration of the response to 0.5 M glycerol (Figure 1C and D), as indicated by the integral of the ratio trace over the course of the response (Supplementary Figure 1). Thus, the presence of food appears to enhance avoidance of soluble repellents by increasing and in some cases by prolonging ASH sensory responses.

Food modulation of ASH sensory responses is mediated by dopamine

Monoamines, in particular dopamine and serotonin, have been implicated in food-related behavioural plasticity in *C. elegans* (Chao *et al*, 2004; Harris *et al*, 2009; Ezak and Ferkey, 2010). We therefore investigated whether dopamine and/or serotonin are involved in mediating the enhancing effect of food on avoidance responses. Previous studies have found that exogenous serotonin and dopamine added to the experimental plates or the buffer can mimic the effects of aminergic signalling on many behaviours (Horvitz *et al*, 1982; Schafer and Kenyon, 1995; Chao *et al*, 2004; Ezak and Ferkey, 2010). Therefore, we performed the drop test on wild-type animals on food-free plates containing 10 mM dopamine or 10 mM serotonin. We observed that exogenous dopamine increased avoidance responses to copper; in contrast, exogenous serotonin did not affect avoidance responses to copper (Figure 2A). We also used calcium imaging to test whether addition of 10 mM dopamine or 10 mM serotonin to the recording buffer affects neuronal responses in ASH. We found that whereas serotonin does not affect ASH calcium transients (data not shown), 10 mM dopamine increases both the magnitude and the duration of responses to 2 mM $CuCl_2$ (Figure 2D and H and Supplementary Figure 1), 10 mM $CuCl_2$ (Figure 2H and Supplementary Figure 1) and 0.5 M glycerol (Figure 2H and Supplementary Figure 1). We reported previously that responses to a non-chemical stimulus, nose touch, are enhanced by exogenous serotonin (Hilliard *et al*, 2005). However, in this study, we could not find any differences between nose touch responses off-food and in the presence of exogenous dopamine or serotonin (Figure 2G and H). Thus, exogenous dopamine mimics many of the effects of food on ASH responses, though some of these (e.g., the prolonging of $CuCl_2$ responses) may be non-physiological.

The modality-specific effects of dopamine on stimulus-evoked calcium transients suggest that dopamine modulation specifically affects sensitivity to chemical repellents rather than the general excitability or synaptic activity of the ASH neurons. To investigate this possibility further, we used a channelrhodopsin 2 (ChR2) transgene expressed specifically in ASH to test the effects of food and dopamine on reversals evoked by non-specific depolarization of ASH. We observed that exposure to blue light evoked reversals in this strain that were dependent on the ChR2 cofactor retinal, indicating that ChR2 could robustly activate ASH. Neither the frequency nor the magnitude of these reversals was detectably affected by the presence or absence of food. Likewise, addition of exogenous dopamine to animals off-food did not enhance ChR2-evoked reversal responses (Figure 3A–D). These results further support the hypothesis that dopamine modulation of ASH is repellent-specific and most likely acts at the level of the sensory response.

Together, these results suggest that endogenous dopamine signalling might specifically enhance ASH responses to chemical repellents in response to food. To test this possibility, we investigated the phenotype of *cat-2(e1112)* animals, which are defective in dopamine biosynthesis (Lints and Emmons, 1999). In behavioural experiments, we observed that *cat-2(e1112)* animals showed indistinguishable avoidance responses to copper in the presence and absence of food (Figure 2B). In contrast, on plates containing 10 mM dopamine, the avoidance response of *cat-2(e1112)* animals was increased (Figure 2B). Thus, endogenous dopamine appears to be necessary for the enhancement of avoidance responses by food. We also analysed ASH neuronal responses in *cat-2* mutants by calcium imaging. In these experiments, neither the magnitude nor the duration of calcium transients

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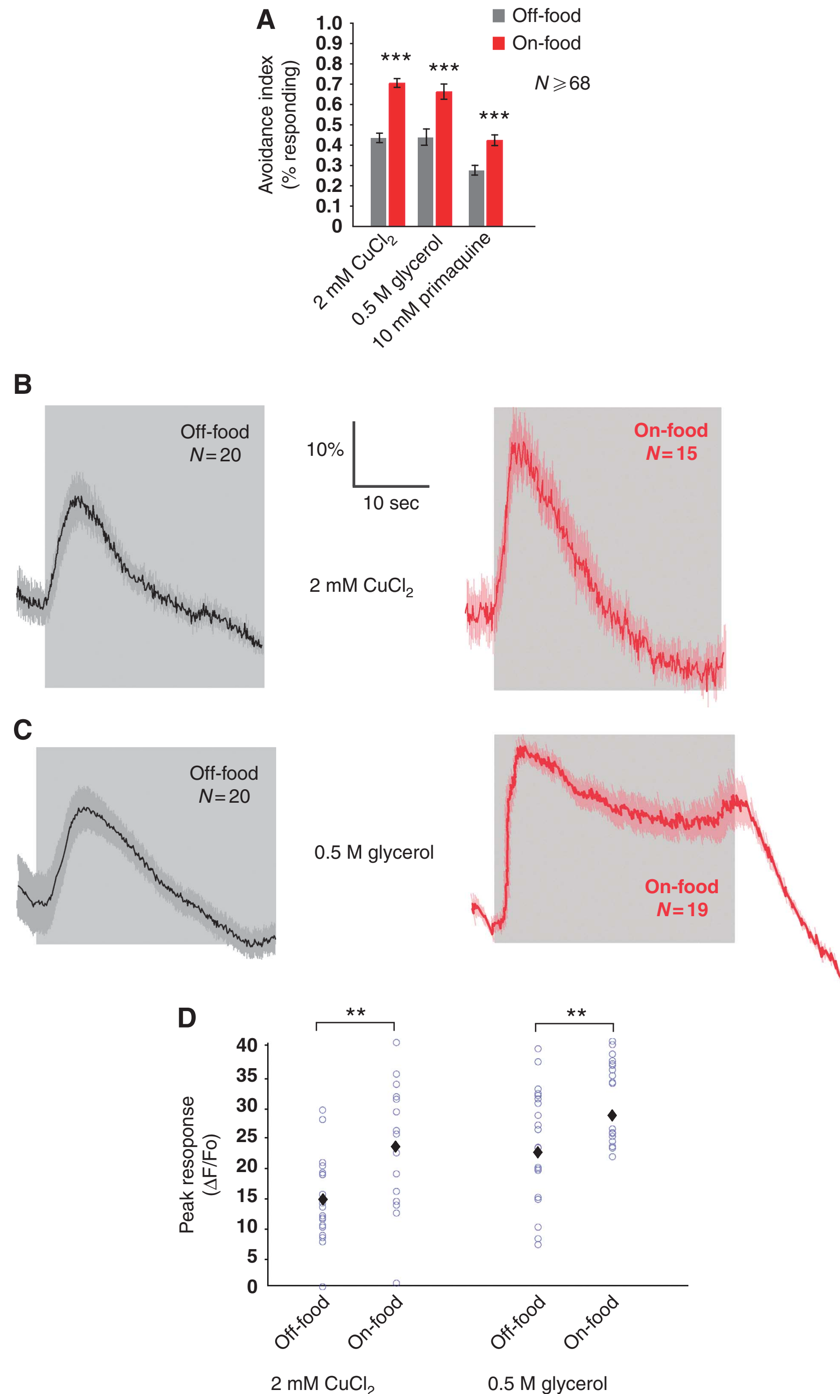

Figure 1 Food enhances taste responses in ASH neurons. (**A**) Avoidance of soluble repellents is increased by food in wild-type animals. Fraction of animals on- and off-food reversing after delivery of a drop with repellent. Avoidance to 2 mM $CuCl_2$, 10 mM primaquine and 0.5 M glycerol is increased on-food. (***$P \leqslant 0.0001$, *t*-test), $N \geqslant 68$. (**B, C**) Average neuronal responses in ASH. Grey shading indicates the duration of the stimulus. (**B**) Neuronal responses to 2 mM $CuCl_2$ are increased by food. (**C**) Neuronal responses to 0.5 M glycerol are increased by food. (**D**) Quantification of ASH responses to 2 mM $CuCl_2$ and 0.5 M glycerol on- and off-food. Blue circles represent individual animals assayed. Black diamonds indicate average value. Food increases the magnitude of ratio changes in wild-type animals. Statistical significance according to the Mann-Whitney test is indicated (**$P < 0.01$), $N \geqslant 15$.

evoked by 2 mM $CuCl_2$ or 0.5 M glycerol were altered by the presence of food (Figure 2E, F and H and Supplementary Figure 1). Together, these results indicate that endogenous dopamine is necessary for food modulation of ASH-mediated avoidance of soluble repellents, and suggest that dopamine signalling directly or indirectly modulates ASH sensory responses generated by food. We also tested whether food affected the ASH sensory adaptation to repeated or continuous stimulation. We showed previously that ASH-mediated avoidance responses attenuate after receiving repeated stimuli or after prolonged incubation in 10 mM $CuCl_2$ (Hilliard *et al*, 2005). We found that in the presence of food, wild-type animals adapt more slowly to repeated stimulation with 10 mM $CuCl_2$ as assayed by the drop test

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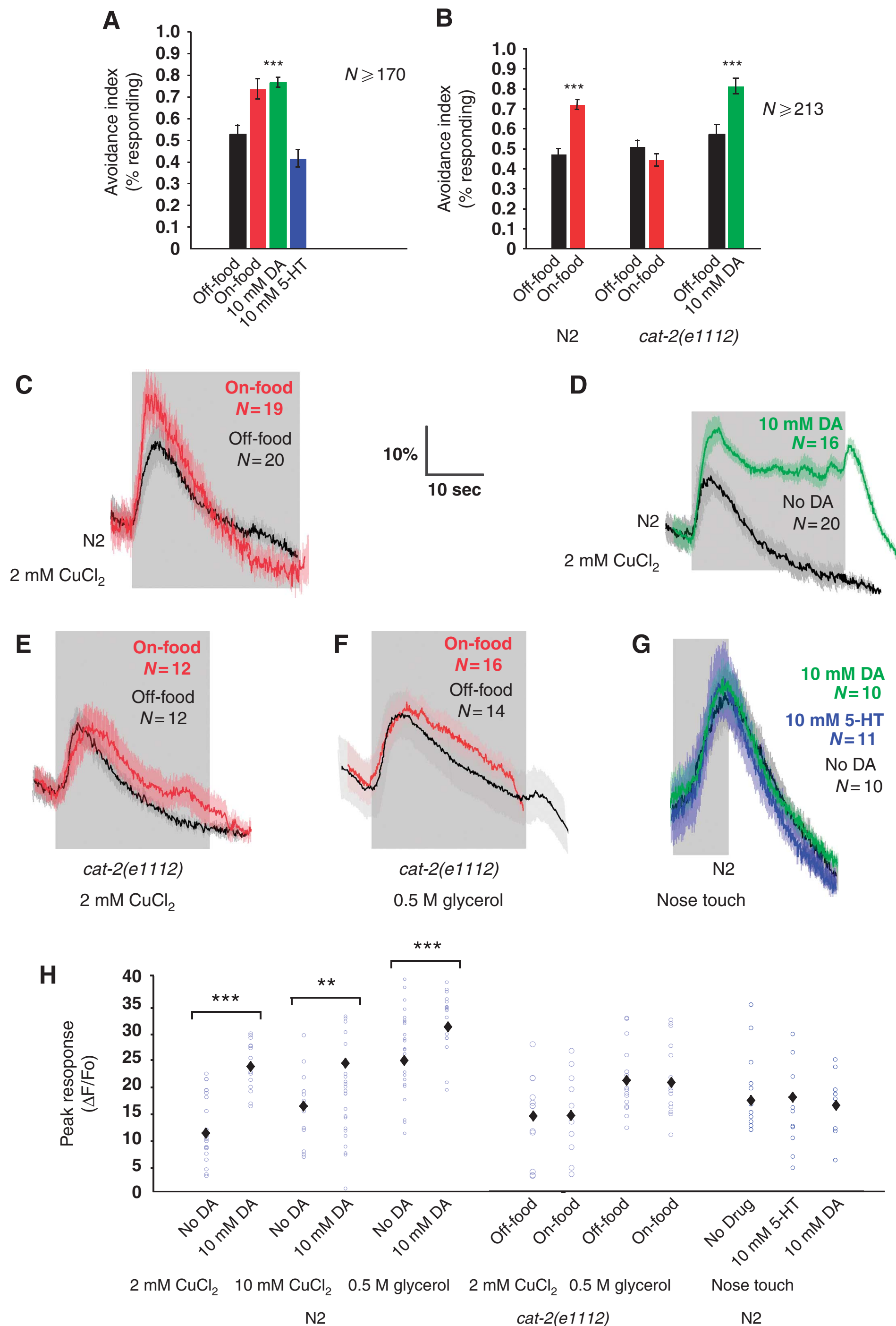

Figure 2 Food modulation of ASH involves dopamine. (**A**) Avoidance is increased by exogenous dopamine (DA). Avoidance is not increased by exogenous serotonin. Fraction of wild-type animals off-food, on-food, on 10 mM DA and on 10 mM serotonin, reversing after delivery of a drop with $CuCl_2$. (***$P<0.0001$ ratio t-test), $N \geq 170$. (**B**) Avoidance is not increased by food but by exogenous DA in *cat-2(e1112)*. Fraction of wild-type and *cat-2(e1112)* animals, on- and off-food, and on and off 10 mM DA, reversing after delivery of a drop with 2 mM $CuCl_2$. $N \geq 213$. (**C–F**) Average neuronal responses in ASH. Grey shading indicates the duration of the stimulus. (**C**) Neuronal responses to 2 mM $CuCl_2$ are increased by food in wild-type animals. (**D**) Responses to 2 mM $CuCl_2$ in wild-type animals are increased by 10 mM exogenous DA. (**E**) Neuronal responses to 2 mM $CuCl_2$ are not affected in *cat-2(e1112)*. (**F**) Neuronal responses to 0.5 M glycerol are not affected in *cat-2(e1112)*. (**G**) Responses to nose touch are not affected by 10 mM serotonin and 10 mM DA in wild-type animals. (**H**) Quantification of ASH responses. Blue circles represent individual animals assayed. Black diamonds indicate average value. Exogenous DA increases the magnitude of the ratio change in wild-type animals. In *cat-2(e1112)*, ASH responses to 2 mM $CuCl_2$ and 0.5 M glycerol are not increased by food. Exogenous DA or serotonin does not affect the ratio change in wild-type animals in response to nose touch. (***$P<0.001$, **$P<0.01$, Mann–Whitney rank sum test), $N \geq 12$.

(Figure 4A). Addition of exogenous dopamine to food-free assay plates slowed habituation to copper, mimicking the effect of food (Figure 4A). Conversely, dopamine-deficient *cat-2* mutants adapted rapidly in the presence of food (Figure 4B), suggesting that endogenous dopamine is required for the inhibitory effect of food on adaptation. We further tested whether food also affects adaptation to continuous stimulation using a different assay (Hilliard *et al*, 2002),

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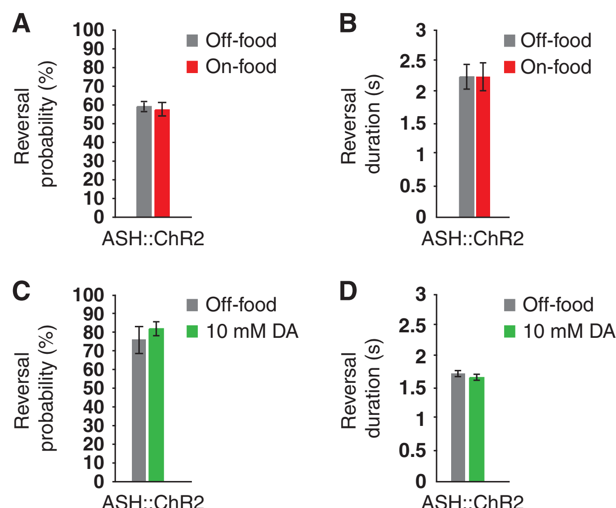

Figure 3 Food and dopamine do not affect reversals caused by optogenetic activation of the ASH neurons. (**A**–**D**) Reversals following optogenetic activation of the ASH neurons. (**A**) Reversal probability is not increased by food. (**B**) Reversal duration is not increased by food. (**C**) Reversal probability is not increased by DA. (**D**) Reversal duration is not increased by DA.

in which the animals are tested after 15 s incubation in 10 mM $CuCl_2$. Also, in these conditions, we found that adaptation in wild-type animals is inhibited in the presence of food (Figure 4C and D), and that *cat-2* mutants adapt rapidly both in the absence and in the presence of food (Figure 4C and D).

To determine whether these behavioural effects of food result from changes in ASH sensory responses, we measured copper-evoked calcium transients in ASH before and after 15 s treatment with 10 mM $CuCl_2$. In the presence of food or exogenous dopamine, ASH responses were significantly larger after the chronic treatment than in animals tested in the absence of food or dopamine (Figure 4E–H). Moreover, in *cat-2* mutants subjected to chronic 10 mM $CuCl_2$ exposure, calcium transients were not affected by the presence of food, and calcium transients were absent in both on-food and off-food conditions (Figure 4E, F and H). Together, these experiments indicate that food decreases adaptation to $CuCl_2$ through dopamine signalling. Our calcium imaging data show that the initial responses also to 10 mM $CuCl_2$ were increased by food and dopamine; however, we could not detect this in our behavioural assays as almost 100% of the tested animals respond to this concentration of copper.

Modulation of avoidance behaviours requires dopaminergic sensory cilia

The dopaminergic neurons of *C. elegans* are mechanosensors that appear to directly sense the presence of a bacterial lawn (Sawin *et al*, 2000; Kindt *et al*, 2007). However, these neurons also receive input from other neurons via synapses and gap junctions, and could therefore in principle respond indirectly to internal signals of food availability. To determine whether the dopaminergic neurons sense and respond to food directly, we investigated whether the dopamine neurons' sensory cilia are required for food modulation of repellent responses. We used a method called FRISSC (functional rescue in single sensory cilia), in which a null mutation in the ciliogenic RFX transcription factor DAF-19C, which is required for the production of cilia (Swoboda *et al*, 2000; Senti *et al*, 2009), is rescued cell specifically, allowing for restoration of ciliogenesis and sensory function in individual neurons in a *daf-19* background (Senti *et al*, 2009). To suppress the dauer-formation phenotype in *daf-19* animals, we also introduced a *daf-12* mutation. As Figure 5 shows, *daf-19;daf-12* animals have defective responses to glycerol. When we restored the cilia of the ASH neurons but not the dopamine neurons using either the *gpa-11* or the *gpa-13* promoter, we observed that responses to glycerol were of equal magnitude in the presence and absence of food (Figure 5). However, when we also restored cilia to the dopamine neurons using a *dat-1::daf-19c(+)* transgene, we observed significantly stronger escape responses in the presence of food than in the absence of food (Figure 5). Thus, the cilia of the dopamine neurons are specifically required for food modulation, indicating that dopamine signalling is activated by external sensory cues.

Dopamine is an acute signal of the presence of food

These genetic and pharmacological data suggest that dopamine is released in the presence of food to modulate avoidance behaviours and ASH activity. However, experiments using dopamine signalling-deficient mutants cannot address the dynamics of the dopamine-mediated food signal. In principle, dopamine signalling could act acutely to indicate the presence of food at a given moment; alternatively, dopamine signalling could act on a longer time scale to represent the animal's experience of food over the course of minutes or hours. To investigate the time scale of dopamine action, we generated transgenic lines expressing ChR2 (Nagel *et al*, 2005a, b) under the *dat-1* (dopamine neuron-specific) and *tph-1* (serotonin neuron-specific) promoters, in a *lite-1(ce314)* background. LITE-1 mediates responses to ultraviolet light, and loss of *lite-1* prevents native light responses (Edwards *et al*, 2008; Guo *et al*, 2009). We grew these animals in the presence or absence of the ChR2 cofactor retinal, and performed the behavioural tests on food-free media after a flash of blue light to activate the dopaminergic or serotonergic neurons, respectively. We observed that upon a 10 s blue-light stimulation of the *dat-1::ChR2* line, the animals' rate of locomotion decreased within seconds, a response similar to the dopamine-dependent slowing response to a bacterial lawn (Sawin *et al*, 2000). In a *cat-2* mutant background, this slowing response was not observed, indicating that the dopaminergic neurons inhibit locomotion speed through dopamine release. Blue-light stimulation of the *tph-1::ChR2* line likewise led to retinal-dependent slowing (data not shown), as expected from previous studies, indicating that serotonin inhibits locomotion. Blue-light exposure did not induce slowing in either line if the animals were grown in the absence of retinal. Next, we investigated the time course of the light-induced slowing of locomotion in the *dat-1::ChR2* line. We found that 60–70 s after the light flash, the speed had returned to its baseline level (Figure 6A). This is in line with other studies showing that within 5 min of being on food, animals display a decreased rate of locomotion (Sawin *et al*, 2000).

We next tested how activation of dopaminergic and serotonergic neurons by ChR2 affected ASH-mediated avoidance responses. We observed that the $CuCl_2$ responses of retinal-grown *dat-1::ChR2* animals were significantly enhanced by blue-light stimulation, whereas animals of the same genotype

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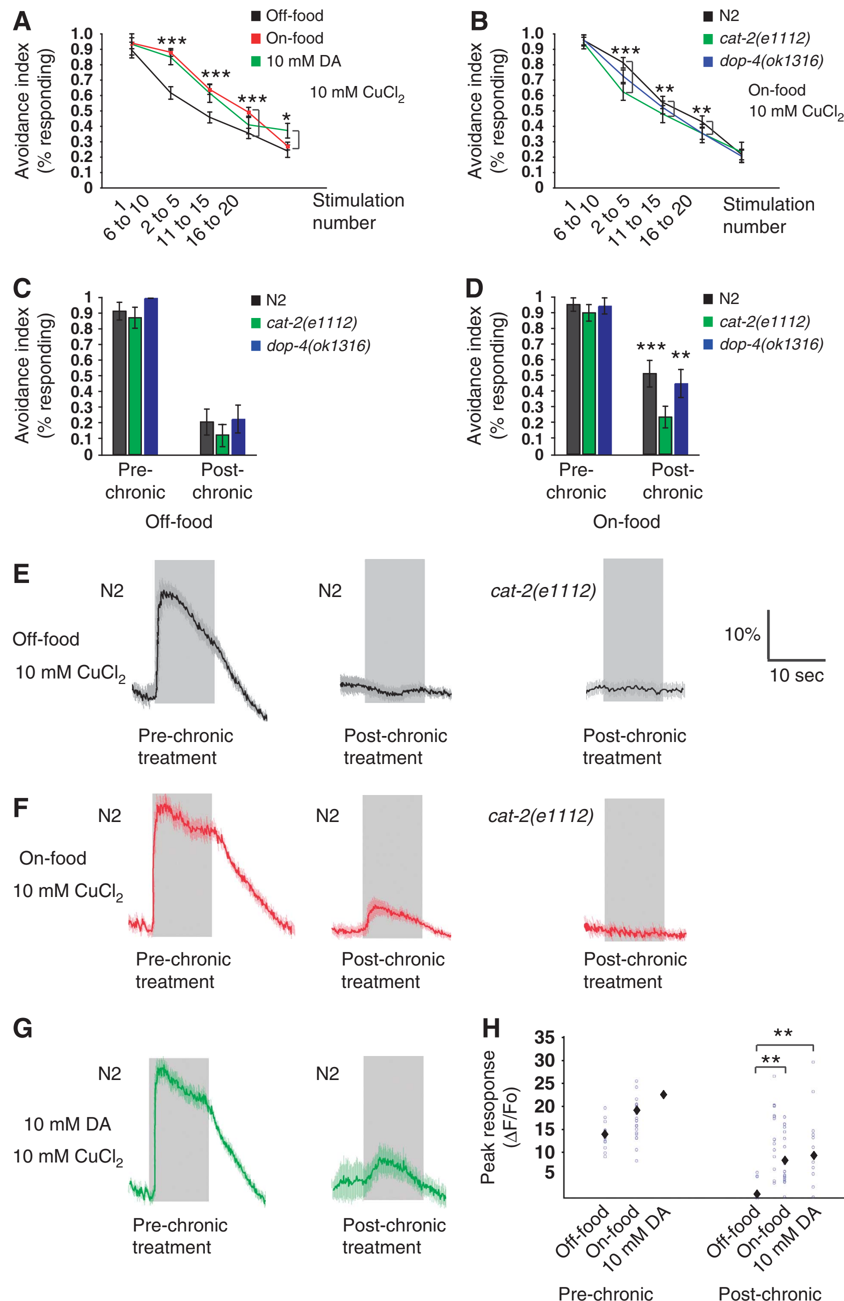

Figure 4 Food and dopamine inhibit adaptation to repellents. (**A**, **B**) Fraction of animals responding to repeated drops of 10 mM $CuCl_2$. (**A**) Wild-type animals adapt slower on-food and 10 mM DA ($P \leqslant 0.036$, *t*-test), $N \geqslant 30$. (**B**) *cat-2(e1112)* animals do not adapt slower on-food. *cat-2(e1112)* adapts faster than wild type on-food, *dop-4(ok1321)* is not significantly different from wild-type animals. (***$P<0.001$, **$P<0.01$, *$P<0.05$, *t*-test), $N \geqslant 37$. (**C**,**D**) Fraction of animals responding before and after a chronic stimulation. (**C**) *cat-2(e1112)* and *dop-4(ok1321)* adapt like wild type to chronic stimulation off-food. (**D**) *cat-2(e1112)* adapts faster than wild type on-food. *dop-4(ok1321)* is not significantly different from wild type. (**E–G**) Average neuronal responses in ASH before and after chronic treatment with 10 mM $CuCl_2$. Grey shading indicates the duration of the stimulus. (**E**) Wild-type and *cat-2(e1112)* animals adapt completely off-food, and show no post-chronic responses. (**F**) On-food, wild type does not fully adapt, and still displays post-chronic responses. *cat-2(e1112)* completely adapts on-food. (**G**) Wild type does not fully adapt and displays post-chronic responses in exogenous DA. ($P<0.001$, *t*-test), $N \geqslant 24$. (**H**) Quantification of ASH responses before and after chronic treatment. Blue circles represent individual animals assayed. Black diamonds indicate average value. Wild type adapts completely to a chronic stimulation with 10 mM CuCl2 off-food, and adapts less on-food or 10 mM DA. ($P<0.01$, Mann–Whitney rank sum test), $N \geqslant 17$.

grown without retinal or *tph-1::ChR2* animals grown with or without retinal showed no enhancement (Figure 6B). Thus, the acute activation of the dopaminergic neurons quickly and robustly enhances avoidance to $CuCl_2$, whereas activation of the serotonergic neurons does not. When we performed the *dat-1::ChR2* photoactivation experiments in a dopamine-deficient *cat-2* mutant background, we did not observe enhanced avoidance in response to blue light. Thus, activation of the dopaminergic neurons appears to modulate ASH responses through dopamine release.

We then investigated the persistence of dopamine-evoked sensitization to repellents. We found that when *dat-1::ChR2* animals were stimulated with $CuCl_2$, a minute after receiving a blue-light flash, their avoidance response was slightly less sensitized than that immediately after stimulation (Figure 6B). By 2 minutes after *dat-1::ChR2* activation, the

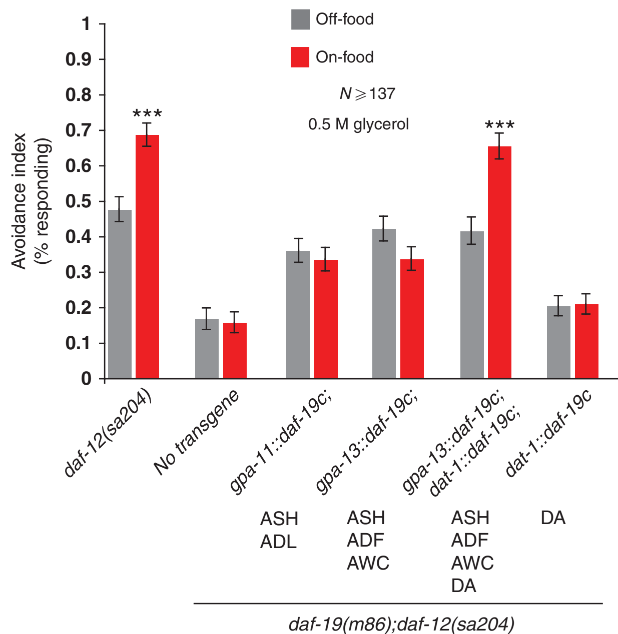

Figure 5 Dopaminergic neurons with intact cilia are required for increased avoidance on food. Fraction of worms reversing on- and off-food after a drop of 0.5 M glycerol. *daf-12(sa204)* animals show increased responses on food, whereas cilia-defective *daf-19(m86);daf-12(sa204)* animals exhibit defective avoidance on- and off-food. Rescuing DAF-19c under the *gpa-11* (ASH, ADL) or *gpa-13* (ASH, ADF, AWC, PHA and PHB) promoters results in normal responses off-food, but does not increase avoidance on food. Rescuing DAF-19c using both the *gpa-13* and *dat-1* (dopaminergic neurons) promoters leads to increased avoidance on food. (***$P<0.001$, *t*-test), $N \geqslant 103$.

$CuCl_2$ avoidance response had returned to its normal off-food level (Figure 6B). These results show a similar time course to the *dat-1::ChR2* effect on locomotion speed. Activation of *dat-1::ChR2* in the presence of food did not further enhance $CuCl_2$ avoidance (Figure 6C), indicating that food and the dopamine neurons affect the same modulatory process. Thus, activation of the dopamine neurons appears to cause an immediate and transient enhancement of ASH-mediated repellent avoidance, suggesting that dopamine signals the acute presence of food in the environment.

The DOP-4 dopamine receptor acts in ASH to enhance repellent responses

To identify the receptor mediating the effects of dopamine on ASH, we screened mutants defective in each of the four dopamine-activated G protein-coupled receptors (GPCRs) in the *C. elegans* genome. We first used the drop test to determine whether deletion mutations in each of these genes (*dop-1*, *dop-2*, *dop-3* or *dop-4*) affected the enhancement of repellent avoidance by food. We observed that responses to copper in *dop-1*, *dop-2* and *dop-3* deletion mutants were still modulated by food, whereas responses to copper in *dop-4* mutants were not (Figure 7A). Copper avoidance in *dop-4* mutant animals was also unaffected by treatment with 10 mM dopamine (Figure 7B) or by photoactivation of the dopaminergic neurons (Figure 6B). Interestingly, *dop-4* did not prevent photoactivation-induced slowing of locomotion (Figure 6A), indicating that it affects only a subset of dopamine-related phenotypes. Thus, DOP-4 is a candidate for mediating the enhancement of ASH responses by dopamine. To test DOP-4 directly, we imaged ASH calcium transients evoked by copper and glycerol in *dop-4* mutants. We found that in *dop-4* mutants, the magnitudes of ASH calcium transients evoked by copper or glycerol were not significantly increased by dopamine or by food (Figure 7C–F). Thus, DOP-4 appears to be important for modulation of ASH by dopamine. Interestingly, dopamine still increased the duration of repellent-evoked calcium transients (Figure 7C and D and Supplementary Figure 1); thus, this effect may involve a dopamine receptor other than DOP-4 or, alternatively, may reflect a non-physiological effect of exogenous dopamine.

A *dop-4::gfp* promoter fusion has been shown to be expressed in a number of neurons as well as non-neuronal tissues, but these do not include the ASH neurons (Sugiura *et al*, 2005). Additional *dop-4* reporter transgenes constructed in our lab likewise did not show visible expression in ASH (data not shown). However, as gfp expression can be below the limit of detection, we used cell-specific rescue and knockdown experiments to determine whether DOP-4 might function directly in ASH. We first generated transgenic animals expressing wild-type *dop-4(+)* under the control of its own promoter and two additional promoters, *sra-6* and *gpa-13*, whose expression patterns overlap only in ASH. Both ASH-expressed transgenes rescued the food-modulation defect (assayed by behaviour) of the *dop-4* deletion mutant, as did expression under the *dop-4* promoter (Figure 8A). In contrast, expressing *dop-4(+)* under a non-ASH promoter (*gcy-5*) did not rescue the mutant phenotype (Figure 8A). The *sra-6::dop-4(+)* transgene also restored the ability of dopamine to enhance repellent-evoked calcium transients in ASH in the *dop-4* deletion mutant (Figure 7C and F). These results suggest that the DOP-4 receptor can act cell autonomously in ASH to mediate dopamine enhancement of repellent responses.

To further test whether DOP-4 functions in ASH for dopamine modulation, we knocked down *dop-4* expression cell specifically using RNAi (Esposito *et al*, 2007). Although transgenic RNAi silencing can spread between *C. elegans* tissues (Jose *et al*, 2009), spreading of cell-specific RNAi has not been observed between different neurons (Esposito *et al*, 2007; Harris *et al*, 2009; Chatzigeorgiou *et al*, 2010). Therefore, we generated transgenes expressing sense and antisense *dop-4* sequences (hence referred to as RNAi transgenes) under the control of the *dop-4* promoter, and the *sra-6* and *gpa-13* promoters. As controls, we also generated RNAi transgenes expressed under the control of the *flp-17* and *gcy-5* promoters, which do not drive the expression in ASH. We observed that *dop-4*, *sra-6-* and *gpa-13*-driven RNAi transgenes led to a defect in dopamine modulation of repellent avoidance (Figure 8B). However, expressing a *dop-4 RNAi* transgene under the *flp-17* and *gcy-5* promoters did not affect dopamine modulation of repellent avoidance. These results are consistent with the cell-specific mutant rescue data indicating that DOP-4 acts cell autonomously in ASH to mediate the enhancement of repellent responses by food and dopamine.

Discussion

In this study, we show that the behavioural escape responses to soluble repellents are enhanced by food, and that this

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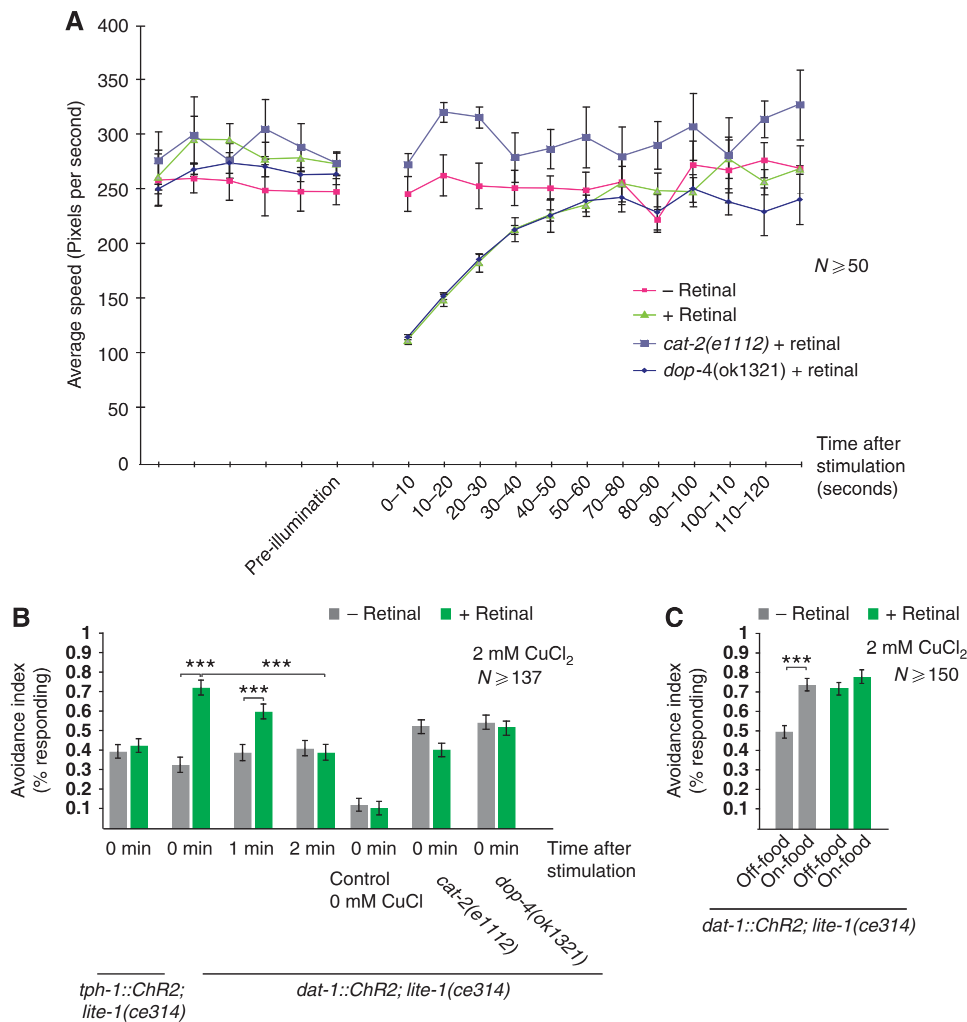

Figure 6 Optogenetic activation of the dopaminergic neurons induces slowing and increases avoidance. (**A**) Normalized speed in animals expressing ChR2 under the *dat-1* promoter (dopaminergic neurons). Shown is the normalized speed measured every 10 s. Animals not grown on retinal are not affected by blue-light stimulation. In animals grown on retinal, a 10-s-long blue-light stimulation induces slowing, which lasts for 60 s. ($P \leqslant 0.0006$, *t*-test), $N \geqslant 50$. Blue-light stimulation does not induce slowing in *cat-2(e1112)*. *dop-4(ok1321)* slows like wild type. (**B**) Fraction of animals reversing after blue-light stimulation, followed by a drop of 2 mM $CuCl_2$. Time between light activation and drop delivery is indicated. Animals not grown on retinal are not affected by the blue-light stimulation. Animals expressing ChR2 under the *tph-1* promoter (serotonergic neurons) and grown on retinal do not have increased avoidance after the light flash. Animals expressing ChR2 under the *dat-1* promoter and grown on retinal show increased avoidance after blue-light activation, and this effect lasts 1–2 min after the light flash. ($P \leqslant 0.0002$, *t*-test), $N \geqslant 137$. Avoidance is not affected in *dat-1::ChR2* transgenes in *cat-2(e1112)* and *dop-4(ok1321)* backgrounds. (**C**) Food does not further enhance avoidance in animals expressing ChR2 under the *dat-1* promoter. Fraction of animals reversing after blue-light stimulation, followed by a drop of 2 mM $CuCl_2$. *dat-1::ChR2; lite-1(ce314)* animals not grown on retinal have enhanced responses on food. *dat-1::ChR2; lite-1(ce314)* animals grown on retinal have enhanced responses both off- and on-food, and the avoidance is not further increased in the presence of food. (***$P<0.001$, *t*-test), $N \geqslant 150$.

enhancement results at least in part from changes in the response properties of the primary chemosensory neurons. Food modulation potentially involves at least three different pathways that increase the sensory responsiveness of the ASH nociceptors. In the first pathway, dopamine acts directly on ASH through dopamine receptor DOP-4 to increase the magnitude of its acute responses to repellents. In the absence of food, dopamine signalling does not affect ASH responses; thus, activation of the dopaminergic pathway functions as a direct 'on-food' signal to facilitate larger sensory responses in ASH. In the second pathway, dopamine acts in a *dop-4*-independent manner to inhibit adaptation to repeated stimuli in the presence of food. Finally, dopamine also acts in a third, *dop-4*-independent pathway to prolong initial responses to repellents such as glycerol in the presence of food. The duration of responses to glycerol in *dop-4* animals are unaffected by the presence of food, and the molecules required for this third pathway remain unidentified. As none of the dopamine-activated GPCRs affect response duration, we speculate that a dopamine-gated ion channel may be involved.

These studies add to the already remarkable number of food-dependent modulatory pathways acting on the ASH neurons. For example, several studies have shown that ASH-mediated avoidance behaviours are also modulated by food through serotonergic signalling. Responses to nose touch are enhanced in the presence of food; this process has been shown to require serotonin signalling and can be mimicked by provision of exogenous serotonin (Chao *et al*, 2004). However, when we tested nose touch responses using

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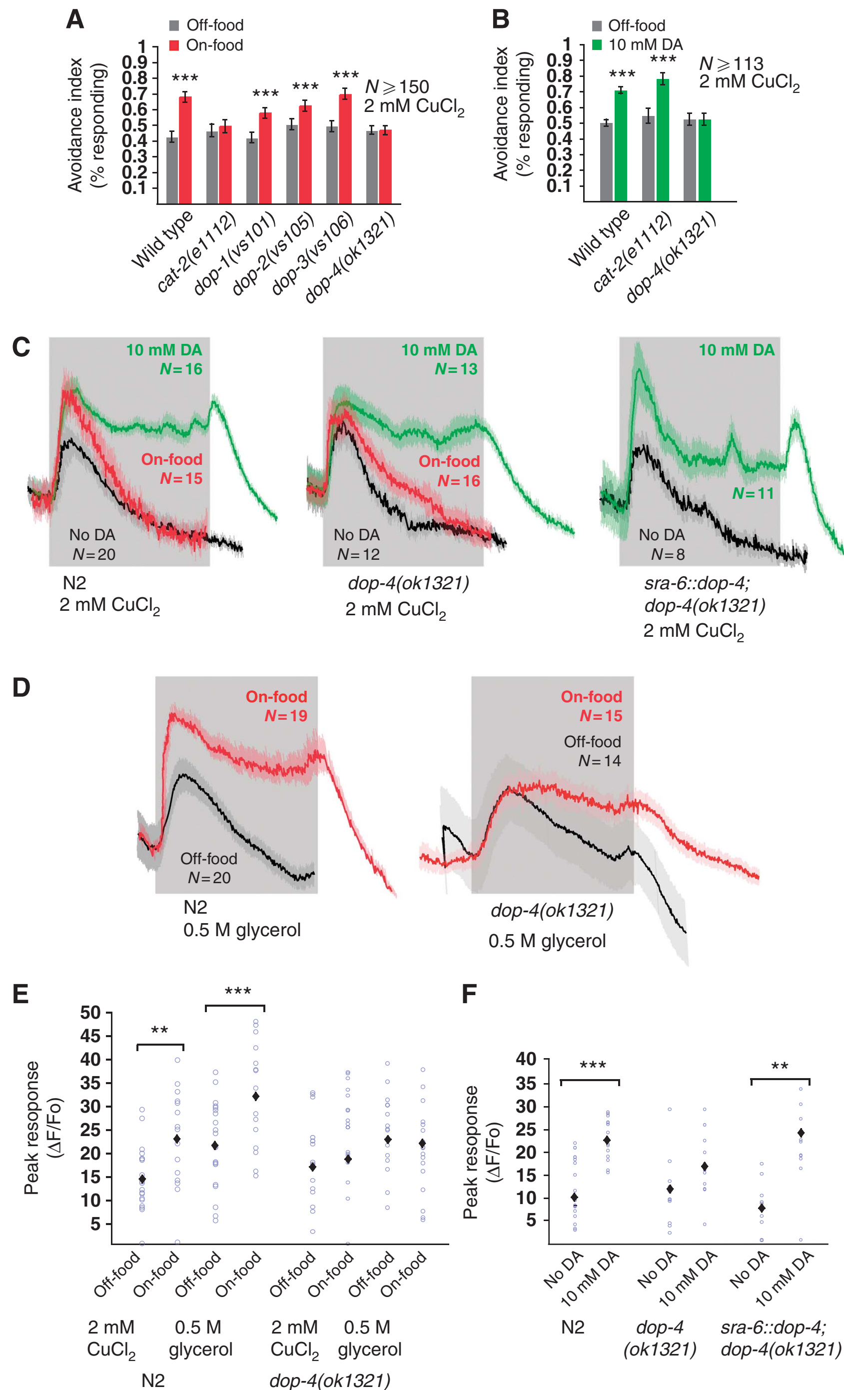

Figure 7 The dopamine receptor DOP-4 mediates the effect of food on avoidance. (**A**) Avoidance is not increased by food in *dop-4(ok1321)*. Fraction of animals responding to 2 mM $CuCl_2$. (***$P<0.001$, *t*-test), $N \geq 150$. (**B**) Avoidance in *dop-4(ok1321)* is not increased by exogenous DA. 10 mM exogenous DA increases the avoidance to 2 mM $CuCl_2$ in wild type and *cat-2(e1112)*, but not in *dop-4(ok1321)*. (***$P<0.001$, *t*-test), $N \geq 113$. (**C**,**D**) Average neuronal responses in ASH. Grey shading indicates the duration of the stimulus. (**C**) Magnitude of ASH responses to 2 mM $CuCl_2$ is not increased by food or exogenous DA in *dop-4(ok1321)*. In wild-type animals, the magnitude of responses to 2 mM $CuCl_2$ is increased by food and 10 mM exogenous DA, but not in *dop-4(ok1321)*. Expression of genomic *dop-4* DNA under the *sra-6* promoter (ASH, ASI, PVQ) rescues ASH responses in *dop-4(ok1321)*. Shown are the average traces. (**D**) Magnitude of ASH responses to 0.5 M glycerol is not increased by food in *dop-4(ok1321)*. In wild-type animals, the magnitude of responses is increased by food, but not in *dop-4(ok1321)*. (**E**, **F**) Quantification of ASH responses. Blue circles represent individual animals assayed. Black diamonds indicate average value. (***$P<0.001$, **$P<0.01$, Mann-Whitney rank sum test) (**E**) Wild-type animals have increased responses to 2 mM $CuCl_2$ and 0.5 M glycerol on exogenous DA. In *dop-4(ok1321)*, the responses are not increased. (**F**) Rescue of *dop-4(ok1321)* under the *sra-6* promoter restores the magnitude of the response to 10 mM DA. (***$P<0.001$, **$P<0.01$, Mann–Whitney rank sum test), $N \geq 9$.

calcium imaging, we did not find exogenous serotonin to have an effect, suggesting that serotonin modulates ASH activity downstream of calcium signalling, possibly at the synapse. Food and serotonin also enhance responses to dilute octanol, a process requiring the activities of the G-proteins GPA-11 (Chao *et al*, 2004) and EGL-30 (Harris *et al*, 2010), and the serotonin receptor SER-5 (Harris *et al*, 2009). Conversely, octopamine appears to inhibit dilute octanol responses in the

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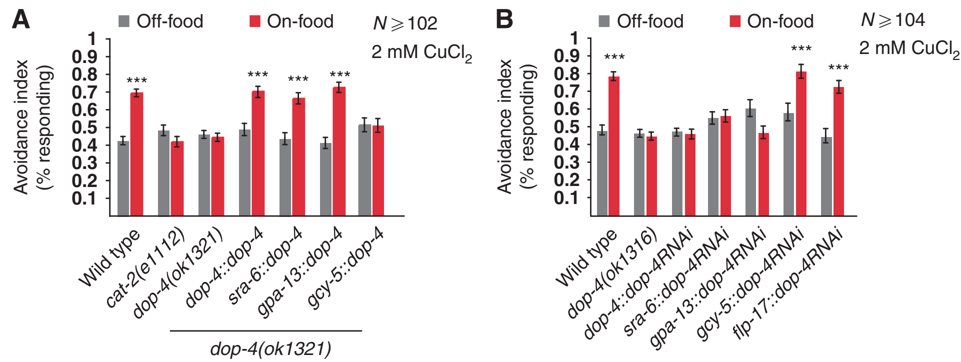

Figure 8 The dopamine receptor DOP-4 functions in ASH to modulate avoidance behaviours. (**A**) Transgenic rescue of *dop-4(ok1321)*. Fraction of animals responding to 2 mM $CuCl_2$. Expressing genomic *dop-4* DNA under its own promoter (ASG, AVL, CAN and PQR), or the *sra-6* (ASH, ASI and PVQ) or *gpa-13* (ASH, ADL, AWC, PHA and PHB) promoters restores the avoidance on food. Expression under the *gcy-5* promoter (ASER) does not rescue. (***$P<0.001$, *t*-test), $N \geqslant 126$. (**B**) RNAi knockdown of DOP-4 in ASH results in defect avoidance on food. Fraction of animals responding to 2 mM $CuCl_2$. Animals expressing *dop-4* RNAi under its own promoter, or under the *sra-6* and *gpa-13* promoters, both of which express in ASH, do not have increased avoidance on food. Animals expressing *dop-4* RNAi under the *gcy-5* and *flp-17* promoters, which do not express in ASH, display wild-type behaviour. (***$P<0.001$, *t*-test), $N \geqslant 94$.

absence of food, a process that requires the OCT-1 receptor and the G-protein GOA-1 (Harris *et al*, 2010). Dopamine does not appear to be involved in enhancing octanol responses in ASH; indeed, exogenous dopamine dampens responses to dilute octanol in a process requiring the activity of dopamine receptor DOP-3 (Ezak and Ferkey, 2010). SER-5, OCT-1 and DOP-3 are required cell autonomously in ASH; thus, including DOP-4, there are at least four different GPCRs that directly modulate ASH activity in response to food. Calcium imaging experiments have not been reported for *ser-5*, *oct-1* or *dop-3* mutants for octanol; thus, it is not clear whether these pathways affect sensory transduction and neuronal excitability, or processes downstream of calcium influx such as synaptic transmission. However, it is clear that the targets of at least two of these pathways differ from those of DOP-4, as neither *dop-3* nor *ser-5* affects the calcium transients evoked by soluble repellents (data not shown). Thus, the molecular mechanisms by which a single environmental condition—food—modulates a single sensory neuron appear to be unexpectedly intricate and complex.

Both DOP-4 and DOP-3 act cell autonomously in ASH; thus, dopamine appears to act directly on the ASH neurons to modulate their activity. *C. elegans* has eight dopaminergic neurons, and activation of this set of neurons is sufficient to immediately induce increased avoidance. On the basis of the EM reconstruction of the *C. elegans* nervous system, the dopaminergic neurons make synapses with a total of 41 neurons; however, these neurons do not include ASH. This implies that dopamine appears to signal extrasynaptically to modulate ASH avoidance responses. Dopamine has been shown to act extrasynaptically in other *C. elegans* neural circuits. For example, the basal slowing response is regulated by DOP-1 and DOP-3 acting in the motor neurons, which receive no significant synaptic input from the dopaminergic neurons (Chase *et al*, 2004). Signalling through the DOP-1 receptor also acts in the ALM anterior body mechanoreceptors to modulate habituation to touch responses (Kindt *et al*, 2007); although the touch receptor neurons synapse onto the dopaminergic CEP neurons, there are no reported synapses from any of the dopaminergic neurons to the ALMs. Indeed, a survey of expression data indicated that of the 118 *C. elegans* neurons expressing dopamine-activated GPCRs, only 20 (17%) were post-synaptic to any of the dopaminergic neurons (Suo *et al*, 2003, 2009; Tsalik *et al*, 2003; Chase *et al*, 2004; Sanyal *et al*, 2004; Sugiura *et al*, 2005; Etchberger *et al*, 2007; Ezak and Ferkey, 2010). The prevalence of extrasynaptic signalling in *C. elegans* is not limited to the dopamine system; only 17 of 45 serotonin receptor-expressing cells are post-synaptic to serotonergic neurons (Tsalik *et al*, 2003; Carnell *et al*, 2005; Xiao, 2006 #2417; Hobson *et al*, 2006; Dernovici *et al*, 2007; Harris *et al*, 2009), and none of the 80 tyramine receptor-expressing neurons are post-synaptic to tyraminergic neurons (Tsalik *et al*, 2003; Rex *et al*, 2004, 2005). This suggests that most aminergic neurotransmission may involve extrasynaptic communication pathways that are not identified in the EM reconstruction of the *C. elegans* anatomy. Thus, even the nominally complete anatomical wiring diagram is likely to significantly underestimate the functional connectivity in the *C. elegans* nervous system.

Dopamine has been implicated in the control of food-dependent states with respect to many behaviours (Sawin *et al*, 2000; Hills *et al*, 2004; Kindt *et al*, 2007; Ezak and Ferkey, 2010). However, the mechanism and dynamics of how dopamine signalling is activated by food to modulate neural circuits has been difficult to discern. We show here that food modulation requires the sensory cilia of the dopamine neurons, indicating that dopamine signalling is directly activated by external sensation of bacteria. Using the light-activated channel ChR2, we found that optogenetic activation of the dopaminergic neurons causes an immediate increase in avoidance responses, an effect that lasts for 1–2 min. This demonstrates that the activation of dopaminergic neurons is sufficient to acutely modulate avoidance responses. Optogenetic activation of the dopaminergic neurons also induces immediate slowing of speed and, interestingly, this effect also lasts between 1 and 2 min, indicating that dopaminergic modulation of both behaviours has a similar time course. This correlates well with the time course of the effects exerted by food itself on behaviour; for example, differences in calcium transients could be observed within a few minutes after the animals are placed on- or off-food. Likewise, the basal slowing response, which requires dopamine, occurs

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within 5 min of being placed on food (Sawin *et al*, 2000), and increases in reversals and turning occur within 1 min of removal from food (Hills *et al*, 2004). Thus, it appears that dopamine is released immediately upon food encounter, and that this signal is only maintained for a prolonged period if the animal remains in a food-rich environment.

Although dopamine itself appears to be a direct, acute signal of food availability, dopamine signalling may interact with other monoamine and neuropeptide systems to integrate information about food and starvation across varying time scales. Previous studies have also indicated that dopamine acts indirectly by controlling the release of other neuromodulators; for example, dopamine appears to negatively regulate release of octopamine from neurons controlling CREB-dependent gene expression in interneurons (Suo *et al*, 2006). Interactions between neuromodulator systems have a critical role in the control of behavioural states in many organisms, including mammals. Future studies of these processes in *C. elegans* may provide insight into conserved mechanisms by which feeding state modifies neural circuits controlling complex behaviours.

Materials and methods

Strains and culture

Strains were maintained as described (Brenner, 1974). All strains were grown at 20°C on NGM plates with OP50. The following strains were used: N2 (wild-type reference), CB1112 *cat-2(e1112)*, LX636 *dop-1(vs101)*, LX702 *dop-2(vs105)*, LX703 *dop-3 (vs106)*, RB1252 *dop-4(ok1321)*, JT204 *daf-12(sa204)*, JT6924 *daf-19(m86); daf-12(sa204)*, OE3789 *daf-19(m86);daf-12(sa204);ofEx588[gpa-13::daf-19c]*, OE3797 *daf-19(m86);daf-12(sa204);ofEx596[gpa-11::gfp; gpa-11::daf-19c;che-13::dsRed;elt-2::mCherry]*, OE3793 *daf-19(m86); daf-12(sa204);ofEx592[dat-1::daf-19c]*. The following strains were generated: AQ2353 *dop-4(ok1321);ljEx241[sra-6::dop-4, unc-122::gfp]*, AQ2548 *dop-4(ok1321)*; *ljEx304[dop-4::dop-4; unc-122::gfp]*, AQ2465 *dop-4(ok1321);ljEx292[gpa-13::dop-4;unc-122::gfp]*, AQ2477 *dop-4 (ok1321);ljEx302[flp-17::dop-4; unc-122::gfp]*, AQ2475 *daf-19(m86); daf-12(sa204);ofEx588[gpa-13::daf-19c];ofEx592[dat-1::daf-19c]*,AQ2483 *ljEx290[sra-6::dop-4RNAi;elt-2::mCherry]*, AQ2519 *ljEx281[gpa-13::dop-4RNAi; elt-2::mCherry]*, AQ2484 *ljEx291[flp-17::dop-4RNAi; elt-2::mCherry]*, AQ2546 *ljEx303[gcy-5::dop-4RNAi;elt-2::mCherry]*, AQ2028 *lite-1(ce314);ljIs100 [Pdat-1::ChR2::yfp,unc-122::gfp]*, AQ2050 *lite-1(ce314);ljIs102 [tph-1;;ChR2::YFP;unc-122::gfp]*, AQ2235 *lite-1(ce314)*; *ljIs114[Pgpa-13::FLPase, Psra-6::FTF::ChR2::YFP]*, AQ2673 *lite-1(ce314)*; *cat-2(e1112)*; *ljIs100[Pdat-1::ChR2::YFP, unc-122::gfp]*, AQ2235 *lite-1(ce314); ljIs114[Pgpa-13::FLPase;Psra-6::FTF::ChR2::YFP]*, AQ2242 *dop-4(ok1321) ljEx95[sra-6::yc2.12, lin-15(+)]*, AQ2180 *cat-2(e1112)*; *ljEx95[sra-6::yc2.12, lin-15(+)]*, AQ2241 *dop-4(ok1321) ljEx95[sra-6::yc2.12, lin-15(+)]*, AQ2303 *dop-4(ok1321) ljEx241[sra-6::dop-4, Punc-122::gfp]*; *ljEx95[sra-6::yc2.12; lin-15(+)]*.

Generation of transgenic animals

Cameleon strains were generated by crossing *lin-15(n765);ljEx95 [sra-6::YC2.12]* (Hilliard *et al*, 2005) with *cat-2(e1112)* and *dop-4(ok1321)*, and by crossing dop-4(ok1321);ljEx95[sra-6::YC2.12] with *dop-4(ok1321);ljEx241[sra-6::dop-4]*. cat-2(e1112) was confirmed by PCR, followed by digestion using StyI. *dop-4(ok1321)* was confirmed by PCR. *daf-19(m86);daf-12(sa204);gpa-11::daf-19;dat-1::daf-19c* was generated by crossing *daf-19(m86);daf-12(sa204); gpa-11::daf-19c* (Senti *et al*, 2009) with *daf-19(m86);daf-12(sa204); dat-1::daf-19c* (Senti *et al*, 2009).

Rescue constructs. Plasmids were constructed using MultiSite Gateway Three-Fragment Vector Construction Kit (Invitrogen). Promoters were inserted into pDONR P4-P1R, except *gpa-13*, which was inserted into pDEST4926. Genomic DNA of *dop-4* was inserted into pDONR 221. A pENTRY P2R-P3 containing the *unc-54* 3′-UTR (kindly provided by Ithai Rabinowitch) was used. Rescue constructs were generated by recombining the pENTRY and pDESTR4-R3 vectors using LR Clonase II Plus. *sra-6* pEntry was kindly provided by Emanuel Busch and contains a 3 kb region of the promoter. *flp-17* pEntry and *gcy-5* pEntry were kindly provided by Andrew Bretscher and contain a 3 kb and 2.2 kb region of the promoter, respectively. The primers used are listed in Supplementary data. *ljEx305*[*sra-6*::*npr-1*], *ljEx304*[*dop-4*::*dop-4*] and *ljEx241* [*sra-6*::*dop-4*] were injected (Mello *et al*, 1991) at 50 ng/μl with co-injection marker *unc-122*::*gfp* at 50 ng/μl. All other rescue constructs were injected at 50 ng/μl with co-injection marker *elt-2*::*mCherry* at 50 ng/μl.

ChR lines. Promoters for *dat-1* and *tph-1* were PCR amplified and cloned into pDONRP4-P1R (Invitrogen). ChR2 (Nagel *et al*, 2003)-coding sequence without a stop codon was PCR amplified from a plasmid MGW16-3 (a kind gift from Emanuel Busch) and cloned into pDONR221 (Invitrogen). These plasmids were recombined in conjunction with a plasmid including YFP and *unc-54* 3′-UTR (a generous gift from Mario de Bono), and pDESTR4-R3 (Invitrogen) using LR clonase II Plus (Invitrogen) to generate pTNZ13 *Pdat-1*::*ChR2*::*YFP* and pTNZ31 *Ptph-1*::*ChR2*::*YFP*. pTNZ13 and pTNZ31 were injected (Mello *et al*, 1991) into wild-type to generate transgenic animals, which were subsequently irradiated with 365 nm UV light at an intensity of 2 mJ/cm^2 in the presence of 0.033 mg/ml trimethylpsoralen (Sigma-Aldrich, MO) to generate integrated transgenic arrays *ljIs100* and *ljIs102*, respectively. Obtained integrants were outcrossed five times with wild type, and then the transgenes were transferred to KG1180 *lite-1(ce314)* to establish AQ2028 (with *ljIs100*) and AQ2050 (with *ljIs102*). FLP recombinase (Davis *et al*, 2008) was used to express ChR2 specifically in ASH by generating pTNZ141(P*gpa-13*::*FLPase*) and pTNZ109(P*sra-6*::*FTF*::*ChR2*::*YFP*), and injecting them at 60 ng/μl and 140 ng/μl, respectively, into *lite-1(ce314)*. The obtained line was irradiated as described above and the integrated strain was outcrossed with *lite-1(ce314)* seven times.

Cell-specific RNAi. The sense and antisense RNAi strands spanning a 1.6 kb region of the *dop-4* gene (Keating *et al*, 2003) were inserted into pDONR221 (Invitrogen). The cell-specific RNAi constructs were generated by recombining the RNAi plasmids with the pENTRY containing promoters for *sra-6*, *gpa-11*, *gcy-5* and *flp-17*, pENTRY containing *unc-54* 3′-UTR, and pDESTR4-R3. The primers used to amplify the RNAi are listed in Supplementary data. For all RNAi constructs, the sense and antisense strand constructs were injected at 100 ng/μl each with co-injection marker *elt-2*::*mCherry* at 50 ng/μl.

Drop test assays

For all behavioural assays, L4 stage animals were picked 20 h before the assay. The drop test and the adaptation drop test were performed on NGM plates and prepared for off- and on-food assays as follows: for on-food plates, 25 μl of overnight culture of OP50 in LB ($A = 0.5$) was spread on each plate. For off-food plates, 25 μl of LB was spread on each plate. All plates were allowed to dry for 1 h without lids, and used after an additional hour for assays. Animals were picked from the culture plate using an eyelash pick, and placed on a plate without food for a few seconds to prevent food from being transferred to the assay plates. The animals were then placed on the assay plate. Animals were allowed to settle for 10 min and then assayed using a capillary to deliver the repellent drop as described previously (Hilliard *et al*, 2002). For the drop test, 10 animals were assayed on each plate. All animals were stimulated every 60 s, and the fraction of worms reversing was recorded. In the adaptation drop test, a single animal was picked to each plate and assayed 20 times every 10 s. The response to every drop was recorded. Responses to chronic stimulation were tested using a previously described protocol (Hilliard *et al*, 2002). A drop of the repellent was first delivered to a single animal to test the pre-chronic stimulation. The animal was then placed in a drop of the repellent for 15 s, followed by a recovery time of 2 min. After the recovery, the animal was tested again for post-chronic responses. The tested repellents were $CuCl_2$ (copper(II)chloride dihydrate, Sigma), glycerol (Fisher) and primaquine diphosphate (Sigma-Aldrich). All repellents were dissolved and diluted in M13 buffer. For behavioural assays with exogenous dopamine and serotonin, fresh assay plates containing 10 mM dopamine or 10 mM serotonin were prepared by spreading dopamine (dopamine hydrocomplex, Sigma-Aldrich) or serotonin (serotonin creatinin sulphate complex,

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Sigma-Aldrich) dissolved in M9 on each assay plate. The same volume of M9, without the drug, was spread on control plates for assays without drugs. All plates were dried without lids for 1 h and used after an additional hour. Dopamine plates were kept in dark until the assay to prevent oxidation. In all serotonin experiments, addition of serotonin stimulated rapid pharyngeal pumping, indicating that it was biologically active.

ChR2 assays

For ChR2 experiments, transgenic animals were grown in the dark at 20°C on NGM plates seeded with OP50 with or without all-*trans* retinal (ATR; Sigma-Aldrich) dissolved in ethanol. For locomotion and drop test assays, adult animals were transferred to an unseeded NGM plate without ATR and left undisturbed for 10 min before illumination. After the recovery, blue light (440–460 nm) from Luxeon III LXHL-PR09 (Lumileds, CA) was used to illuminate the entire plate for 10 s during the recording in order to activate ChR2. The animals were then tested for either speed or avoidance.

To grow animals for speed assays, 5 μM ATR was used and 20 adult animals were tested in each experiment. Movies were recorded with a USB-connected camera AM413TL (AnMo, Taiwan) at the frequency of 2 frames per second, and analysed using Parallel Worm Tracker (Ramot *et al*, 2008) running on MATLAB (Mathworks, MA). The speed of all the animals in the movie frame was averaged to generate one speed value at each time point. Assays were done for 11 different sets of animals for each condition (with/without ATR). For avoidance assays, 62.5 μM ATR was used to grow the animals, and 10 adult animals were stimulated with repellent drops as described for the drop test. The drop was delivered 0, 1 or 2 min after the illumination. As a control for reversals caused by the ChR activation, the same assay was performed delivering drops with only M9 buffer. To test for reversals following ChR activation of ASH, adult animals were transferred to plates that were unseeded or seeded, or that containing 10 mM dopamine. The animals were allowed to recover for 5 min and illuminated for 1 s. Reversals were analysed using the Parallel Worm Tracker (Ramot *et al*, 2008).

Calcium imaging experiments

Sample preparation and delivery of repellent. Animals were glued on 2% agarose pads made with neuronal buffer using cyanoacrylate glue (Nexaband S/C, Abbott Laboratories). The pad was briefly placed on ice during the gluing to constrict the animal's movements. The animals were placed under the microscope in a perfusion chamber (RC-26GLP, Warner Instruments) under constant flow rate (0.4 ml/min) of neuronal buffer using a perfusion pencil (AutoMate). Outflow was regulated using a peristaltic pump (Econo Pump, Biorad). Repellents were delivered using the perfusion pencil and automated valves (EW-98302-20, Cole Parmer Ltd), controlled by Motorway software 2.5. The neuronal buffer contained 40 mM NaCl, 10 mM HEPES-NaOH pH 7.1 and 1 mM $MgSO_4$, and the osmolarity was adjusted to 350 mOsmo using glycerol. Copper and glycerol were dissolved in M13 buffer. For recordings with food, OP50 was grown overnight in LB ($A=0.5$), and 50 ml cultures were pelleted by centrifugation. The pellets were dissolved in neuronal buffer or repellent, to a final volume of 25 ml, and used within 1 h.

Calcium imaging and image analysis. Optical recordings were performed on a Zeiss Axioskop 2 upright compound microscope using a ×63 Zeiss Achroplan water immersion objective. Filter/dichroic pairs were: excitation, 420/40; excitation dichroic 455; CFP emission, 480/30; emission dichroic 505; YFP emission, 535/30 (Chroma). The microscope was fitted with a Hamamatsu Orca ER CCD camera, a Hamamatsu W-View emission image splitter and a Uniblitz Shutter (Vincent Associates). Images were acquired at 10 Hz using MetaVue 4.6 (Universal Imaging). Image analysis was performed using a custom programme written in Java parameterized and averaged using scripts written in Matlab 6.5.1 (Mathworks).

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (http://www.embojournal.org).

Acknowledgements

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Conflict of interest

The authors declare that they have no conflict of interest.

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